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Larval Development, Metamorphosis, and Juvenile Feeding of *Doridella steinbergae* (Lance) (Opisthobranchia: Nudibranchia)

by

© LOUISE ROBERTA BICKELL

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA
SPRING, 1978

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Larval Development, Metamorphosis, and Juvenile Feeding of Doridella steinbergae (Lance) (Opisthobranchia: Nudibranchia)" submitted by Louise Roberta Bickell in partial fulfilment of the requirements for the degree of Master of Science.

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DEDICATION

I dedicate this thesis to my mother and father.

ABSTRACT

A study of the life history of Doridella steinbergae (Lance), a dorid nudibranch which lives and feeds on epiphytic colonies of the bryozoan, Membranipora villosa, was carried out. D. steinbergae was found to exhibit the characteristics of a seasonal nudibranch. According to Thompson (1964), these characteristics facilitate maximum exploitation of a transient food source.

Following an embryonic period of 10 to 12 days at 9 - 10°C, the veligers hatch from the egg mass and require a planktotrophic period of 26 to 37 days (depending on the laboratory culture conditions) to attain metamorphic competence. Colonies of M. villosa encrusted on either algae or glass slides, and seawater conditioned with M. villosa induced metamorphosis of competent veligers. Veligers exhibited a preference for the periphery of the bryozoan colony as the site of metamorphosis.

Larval development was arbitrarily divided into four phases and histological examination was made of each of these. Developmental events involving the foot, alimentary tract, nervous system, larval kidney complex, larval heart, gonadal rudiment, mantle fold, and muscle systems are described.

Histological investigation of the metamorphic process was also performed. It is proposed that three main processes produce the reorganization of the body plan at metamorphosis. These are: contraction of the larval retractor muscle, loss of the shell and operculum, and secretion of adhesive material by the multicellular pedal glands. Study of the metamorphic process has provided an explanation for the subnotal position of the anus in this corambid nudibranch. This explanation is contingent upon the uncoupling of the process of mantle fold hypertrophy from mantle fold reflection. Emphasis is placed on the metamorphic transformations of the alimentary tract. The major events are the loss of the entire dorsal stomach and most of the ventral stomach, differentiation of the salivary glands and buccal pump, extensive muscularization of the gut, and differentiation of zymogen cells in the digestive gland.

Ontogenetic change in the method of feeding on _M. villosa_ zooids was observed. Young juveniles, which measure from 1/3 to 1/5 the length of the bryozoan zooecium, are able to feed on zooids by dissociating the cells of the prey prior to ingestion. Juveniles abandon this method of feeding at approximately 13 days of age.

D. steinbergae grows and matures rapidly after metamorphosis; sexual maturity is attained at 22 to 26 days of age. It is suggested that the differentiation and growth of the primary germ cells during the larval stage may occur at the expense of larval shell growth, and the phenomenon may facilitate rapid attainment of sexual

maturity during the benthic stage of the life cycle.

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INTRODUCTION

Nudibranchs are characterized as gastropods which have lost the shell, mantle cavity, and ctenidia, and possess rhinophores in place of tentacles. Of the various orders within the Opisthobranchia, the nudibranchs display the greatest degree of detorsion (Morton, 1960).

Functionally, nudibranchs are distinguished by their exclusively carnivorous diet (Forrest, 1953; Morton, 1960; Thompson, 1964). Sessile organisms such as cnidarians, ascidians, barnacles, sponges, and bryozoans constitute the prey of these epifaunal grazers (Forrest, 1953; Aboul-Ela, 1959; Sweenen, 1961; Thompson, 1964; McBeth, 1971). Thompson (1964), has indicated that the members of each family tend to feed on similar types of prey. For example, the Eubranchidae are hydroid-feeders, the Glossodoridae are sponge-feeders, and the Limaciidae feed on bryozoans. While some nudibranchs will accept a broad range of prey species, others will select only a single genus or species of prey (Thompson, 1964). In the latter case, the entire, post-metamorphic life is spent in association with the food organism. Harris (1973) has even expressed the view that the association between nudibranchs and their prey is a type of symbiosis.

Nudibranchs exhibit specialized morphological and behavioral adaptations for the efficient exploitation of their respective prey. Thompson (1960; 1964) has found a correlation between the general form of the nudibranch and the form of the prey. Those which feed on flat, encrusting organisms tend to have a broad, oval foot and are cryptic against the background of the prey. Nudibranchs which feed on vertically oriented hydroids and arborescent bryozoans, tend to have a long, narrow foot and possess brightly coloured papilla which make them conspicuous. The two categories of foot shape relate to maximum maneuverability on the prey. Thompson (1960), attempted to justify the presence or absence of crypsis in terms of differing defense strategies.

In each nudibranch species, the structure of the gut, and particularly of the buccal apparatus, is designed for efficient manipulation and processing of the respective type of prey. Detailed studies on the functional morphology of the alimentary tract have been performed on a number of nudibranch species by Millot (1937), Graham (1938), Forrest (1953), Thompson (1962), and Morse (1967).

In addition to morphological correlates, Miller (1962) and Thompson (1964) have found a relationship between the life cycles of nudibranchs living in temperate waters, and those of their prey. Annual nudibranchs tend to feed on organisms which form stable, abundant populations throughout the year. The life history of these species is characterized by slow growth and a single, yearly spawning season. Seasonal nudibranchs attack fast-growing organisms of transient abundance. These species grow to sexual maturity rapidly and pass through a number of generations per year.

Nudibranchs are hermaphroditic and fertilize internally; this is typical of all members of the Opisthobranchia (Morton, 1960). The spawn is attached to the substrate, and consists of a number of ova-containing egg capsules which are packaged in various ways within a jelly-like matrix (Hurst, 1967). The embryogenesis of various opisthobranchs has been described in extensive detail by Casteel (1904), Saunders and Poole (1910), Pelseener (1911), Thompson (1958), and Raven (1958). In typical molluscan fashion, cleavage is spiral and determinate. The initial cleavages produce a stereoblastula which gastrulates by invagination (Casteel, 1904), by epiboly (Thompson, 1958; 1962) or by both methods (Rao, 1960; Smith, 1967; Williams, 1971). The blastopore soon closes and the shell gland invaginates at the posterior end of the embryo.

Subsequent morphogenesis produces the velum, foot, and shell. A single or, in at least one case, a paired (Bonar and Hadfield, 1974) larval retractor muscle differentiates, as well as a nervous system consisting of a variable number of ganglia.

The gut is formed by the progeny of the entoblast cells (Casteel, 1904). In the aeolid, Fiona marina

(Casteel, 1904), the dendronotid, Tritonia hombergi (Thompson, 1962), and the anaspidean, Aplysia californica (Saunders and Poole, 1910), the anal cells differentiate postero-ventrally, slightly to the right of the midline. Later in development, these cells migrate anteriorly to their final destination on the right lateral side of the embryo. Thompson (1962) states that this migration is the only remaining vestige of mechanical torsion. In the dorid, Adalaria proxima, Thompson (1958) found no migration of the anal cells; their position at differentiation was at the definitive, right lateral location (90° torsion.).

Following the early embryonic events in opisthobranchs, there is considerable variation in subsequent morphogenesis. A number of classification schemes have been proposed to organize this diversity of developmental patterns.

Shape differences of the larval shells was used in the first classification scheme (Vestergaard and Thorson, 1938). The shells were grouped into three types: cap-shaped (type A), spiral (type B), and inflated (type C). However, Thompson (1961), and subsequently Hurst (1967), eliminated the cap-shaped category, and renamed types B and C as types 1 and 2, respectively. This modification was justified with evidence that cap-shaped shells are merely the result of abnormal embryogenesis. Type 1 shells are found in all dorids and some aeolids, while type 2 shells are found only in certain dendronotid and aeolid families (Thompson, 1961; Hurst, 1967; Williams, 1971). It has been noted that all members of each nudibranch family possess the same type of larval shell (Thompson, 1961; Hurst, 1967).

Thompson (1967), subsequently presented a second classification scheme based on functional, rather than strictly morphological criteria. This system, which was designed to include all opisthobranchs, categorizes development as planktotrophic, lecithotrophic, or direct. Planktotrophic development is the most prevalent pattern among opisthobranchs (Hurst, 1967; Thompson, 1967; Tardy, 1970; Bonar, 1978). However, the initial research on opisthobranch development focused mainly on species belonging to the lecithotrophic and direct categories. The most detailed of these investigations include those of Thompson (1958; 1962) and Bonar and Hadfield (1974) for lecithotrophic species, and Thompson (1967), Smith (1967), Tardy (1970), Morse (1971), Bridges (1975), and Usuki (1967) for direct developers. A more complete list of these studies is given by Bonar (1978).

Franz (1971) provided the first published account of the successful rearing of a planktotrophic opisthobranch veliger. However, the tenure of larval life in the species studied was short, only 8 to 9 days, and the structure and development of the larvae were only superficially described. Study of the life history of long-term planktotrophs has recently been made possible by the development of successful techniques for larval culture (Franz, 1975; Kriegstein, et al., 1974; Switzer-Dunlap and Hadfield, 1977).

Bonar (1978) devised a third classification of opisthobranch development which is a revision of that proposed by Thompson (1967). However, Bonar (1978) recognized that, in terms of morphogenetic considerations, the category of direct development actually encompasses two distinct developmental types. In some of these species, a transient veliger stage, which metamorphoses prior to hatching, is formed within the egg capsule. In other species, the embryo develops directly into a juvenile, without representation of the veliger stage. Therefore, Bonar (1978) proposed that opisthobranch development be considered as either metamorphic or ametamorphic. Within the metamorphic category are the subgroups: lecithotrophic, planktotrophic, and capsular metamorphic development (metamorphosis from larva to juvenile occurring within the egg capsule).

The most elaborate categorization of developmental patterns has been proposed by Tardy (1970). His scheme is only applicable to nudibranchs. Like Bonar (1978), Tardy (1970) incorporated the metamorphic process in his organization of nudibranch development. However, in an attempt to reflect possible phylogenetic trends within the order, considerable emphasis is placed on specific events which occur at metamorphosis. These are: the derivation of the definitive, dorsal epidermis of the adult and the post-metamorphic position of the anus. Planktotrophy and lecithotrophy are of secondary importance in Tardy's (1970) scheme.

From a morphogenetic standpoint, a major difference between the classification of Bonar (1978) and that of Tardy (1970), is that Bonar (1978) believes the presence or absence of a recognizeable veliger stage is the criterion of primary importance for distinguishing developmental types. Tardy's (1970) scheme indicates that ametamorphic development is merely a subgroup of veligers having lecithotrophic development.

Unlike the pelagic veligers of prosobranchs, the velar lobes of opisthobranch larvae, with the exception of the dorid, Aegires punctilucens (Thiriot-Quievreux, 1977), are small and are not elaborated into secondary loops (Thorson, 1946; Bonar, 1978). Thorson (1946) used this fact to support his belief that nudibranch veligers are non-feeding, non-growing, and short-lived. Indeed, Thompson (1958; 1962) found that the veligers of Adalaria proxima and Tritonia hombergi, respectively, become competent to metamorphose at one to two days after hatching and do not require food during pelagic life. However, Thompson (1959) recognized that most nudibranch larvae, unlike A. proximа and T. hombergi, are obligate planktotrophs. In a study of feeding and digestion in a number of planktotrophic nudibranch veligers, Thompson (1959) did not mention shell growth or veliger development in any of the species.

Hadfield (1963) attempted to clarify the "feeding - growth - length of larval life interaction" in nudibranch larvae. By using antibiotics, Millipore-filtered sea water, and Monochrysis sp. (a unicellular, golden-brown algae) as the food source, Hadfield (1963) was able to culture Aeolidiella glauca for 36 days, Onchidoris fusca for 32 days, and Eubranchus exiguus for 3 days. The shells of the former two species eventually grew to double their volume at hatching. Although attempts to induce metamorphosis in these species were unsuccessful, Hadfield's (1963) study provided evidence that some nudibranch veligers are not short-lived and these species may grow considerably during the larval phase.

Within recent years, a number of studies have added greatly to our knowledge of larval morphogenesis in planktotrophic species. These include the investigations of Kriegstein (1977; 1977b), Switzer-Dunlap and Hadfield (1977), and Switzer-Dunlap (1978) on a number of aplysiids, Thiriot-Quievreux (1977), Perron and Turner (1977), and Chia and Koss (1978) on dorid nudibranchs, and Kempf and Willows (1978) on dendronotid nudibranchs. However, none of these studies, except that of Kriegstein (1977), included histological information regarding the sequential phases of larval development. Nevertheless, the results of these studies indicate that morphogenesis in planktotrophs is similar to that which takes place within the egg capsule in lecithotrophic and capsular metamorphic species (Bonar, 1978). In both cases, the nervous system eventually develops five pairs of ganglia (cerebrals, pleurals, pedals, optics, and buccals), eyespots appear, the mantle fold retracts from the aperture of the shell, a propodial swelling develops on the foot, and a number of adult structures appear in rudimentary form.

Thompson (1958), in a study of the life history of Adalaria proxima, was the first investigator to show metamorphic induction of an opisthobranch veliger by the prey species of the post-metamorphic phase. This phenomenon has subsequently been demonstrated in other lecithotrophic species (Thompson, 1962; Hadfield and Karlson, 1969) and in a number of species having planktotrophic veligers (Tardy, 1962; Harris, 1975; Kriegstein et al., 1974; Switzer-Dunlap and Hadfield, 1977; Perron and Turner, 1977; Chia and Koss, 1978; Kempf and Willows, 1978). The adaptive value of this selection of the metamorphic site relates to both the prey specificity exhibited by opisthobranchs, and the patchy distribution of the prey in nature (Thompson, 1964).

Hadfield and Karlson (1969) and Hadfield (1972;

1977) have taken a mechanistic approach in the study of metamorphic induction in Phestilla sibogae, an aeolid nudibranch. Metamorphosis in this species is induced by the stony coral Porites compressa. In a review of his research, Hadfield (1978) organized the metamorphic process into four categories: 1. the nature of the metamorphic inducer, 2. tissue competence to undergo metamorphosis, 3. the mode of action of the inducer, and 4. the morphological metamorphic responses. With respect to the first category, Hadfield and Karlson (1969) have shown that extracts of Porites will induce metamorphosis as effectively as the coral itself. The inducing molecule has a molecular weight of less than 500, is stable over a wide range of pH and temperature, and is effective in minute concentrations (Hadfield, 1978). Tissue competence is presently a poorly understood phenomenon in invertebrate larvae. Hadfield (1978) suggested that, in molluscs, competence involves both morphological and neurological components. In addition, Hadfield (1978) gives evidence that the effect of the inducer (its mode of action) may be mediated by the nervous system of the competent veliger. Metamorphosis in Phestilla sibogae will occur in the presence of actinomycin D, puromycin, and cycloheximide. This suggests that de novo synthesis of macromolecules, in response to the inducing molecule, is not involved in the larval response (Hadfield, 1978).

The final aspect of metamorphosis, the morphogenetic changes which convert the pelagic larva into the benthic juvenile, have been described for a number of opisthobranchs. However, due to previous difficulties in rearing planktotrophic larvae through metamorphosis, all the intensive studies have been performed on lecithotrophic and capsular metamorphic species (Thompson, 1958; 1962; Bonar and Hadfield, 1974; Tardy, 1970). In addition, superficial descriptions of metamorphosis, as it was viewed externally, have been provided by Tardy (1962; 1964), Horikoshi (1967), Thiriot-Quievreux (1970; 1977), Kriegstein et al. (1974), Harris (1975), Franz (1971), Perron and Turner (1977), Switzer-Dunlap and Hadfield (1977), Switzer-Dunlap (1978), Kempf and Willows (1978), and Chia and Koss (1978). In a review of this literature, Bonar (1978), states that, among opistobranchs, the metamorphic changes exhibited by nudibranch veligers are the most catastrophic. The conspicuous changes include: loss of the velum, loss of the larval shell and operculum, and dorso-ventral flattening of the body. Histological (Thompson, 1958; 1962; Tardy, 1970) and ultrastructural (Bonar and Hadfield, 1974; Bonar, 1976) investigations also reveal that the internal metamorphic changes include detorsion of the alimentary tract, elimination of visceral flexure, autolysis of the larval retractor muscle, formation of the definitive dorsal epidermis, and continued differentiation of the rudiments of adult structures.

Interest in the metamorphic changes of the larval alimentary tract has centered mainly on the process of detorsion (Thompson, 1958; 1962; Tardy, 1970; Bonar and Hadfield, 1974; Kriegstein, 1977b). Although Thompson (1958; 1962) described the pre- and post-metamorphic structure of the gut in two lecithotrophic species, no studies have been performed on possible tissue transformations of the gut in planktotrophic species.

Doridella steinbergae belongs to the dorid nudibranch family, Corambidae. The characters which describe this family are given by Fischer (1883) (translated by MacFarland and O'Donoghue, 1929) as follows:

> "Body, notaeum, and rhinophores doridoform, branchiae posterior, below the notaeum margin and above the foot; anus median, posterior, between notaeum and foot; reproductive openings anterior on right side; radula narrow, multiserial."

There has been some confusion concerning the taxonomic classification of the family Corambidae. Much of this has resulted from duplicate naming of the family, genera, and species. The first described nudibranch within this taxon was Hypobranchiaea A. Adams 1847. In 1871, Bergh described the genus Corambe and erected the family Corambidae to contain it. Verrill (1870) subsequently gave a brief description of a structurally similar nudibranch which he named Doridella obscura. Fischer (1883) felt the correct name of the family should be Hypobranchiaedae, in accordance with the first described member. This designation has been followed by several later authors (MacFarland and O'Donoghue, 1929; Harry, 1953). Neverthless, Bergh (1892) retained the family name of Corambidae in his system of nudibranch classification and all recent literature recognizes Bergh's nomenclature (Lance, 1962; Franz, 1967; Kozloff, 1974).

The genus, Doridella, has also had a confused taxonomic history since the first description by Verrill (1870). Unlike Corambe, which has a deep notch in the posterior edge of the notum, the notum of Doridella is entire. Balch (1899) described an unnotched corambid from Long Island Sound, but did not recognize it as a species of Doridella. He created the genus Corambella. Several subsequent species were described, all bearing the generic name Corambella. Franz (1967), in a complete re-evaluation of the unnotched corambids, confirmed that Doridella was the correct generic name. He also discarded several of the later described species as being synonymous with earlier ones. At present, three species of the genus, Doridella, are recognized. These are: D. obscura Verrill 1870, recorded from numerous localities along the east coast of the United States; D. carambola (Marcus), found off the coast of Brazil; and D. steinbergae (Lance) which has a range from Baja, California to Vancouver Island.

Doridella steinbergae, in conformance with the generic characteristics, has a broad, oval foot which is completely overlapped by an unnotched dorsal notum (Fig. 1a). There are 3 to 6 branchiae on either side of the anus which is located at the posterior of the animal between the notum and foot. D. steinbergae is distinguished from the other two members of the genus by its long, smooth rhinophores.

A notable feature of the notum of corambids is the presence of a thin, spiny cuticle which is periodically shed (MacFarland and O'Donoghue, 1929; Perron and Turner, 1977). Subsequent elaboration of a new cuticle is performed by special secretory cells in the notal epidermis.

Members of the Corambidae are exclusively associated with species of encrusting bryozoans, particularly those belonging to the family, Membraniporidae. The habitat of Corambe pacifica, as reported by MacFarland and O'Donoghue (1929), is upon Macrocystis, Nereocystis, and Zostera bearing encrustations of Membranipora villosa. O'Donoghue (1926) described juveniles, measuring less than one millimeter, within empty zooecia from which they had apparently eaten the zooid. The older individuals always attacked the growing edge of M. villosa colonies which O'Donoghue (1926) attempted to justify by stating that the growing edge is "either not protected by a chitinous covering, or else by one so thin that it affords no protection".

Franz (1967) found Doridella obscura to be very common in the intertidal zone on shells encrusted with

Membranipora crustulenta. After the autumn die-off of M. crustulenta, the same author found specimens of D. obscura associated with two other species of encrusting bryozoans, Alcyonidium verrilli, and Acanthodesia tenuis.

According to Lance (1962), Marcus (1955) found D. carambola on an unspecified epiphytic bryozoan.

D. steinbergae has been found only on Membranipora sp. (McBeth, 1968; Kozloff, 1973; Seed, 1976). The pigmentation pattern of the nudibranch closely resembles the colonies of this bryozoan (Lance, 1962). The otherwise translucent notum bears a latticework of white reticulating lines and occasional irregular blotches of orange (Fig. 1a). When on its natural substrate, the nudibranch is almost undetectable (Fig. 1b).

Along the coast of southern California, Lance (1962) reported that populations of D. steinbergae on a single kelp frond may comprise as many as sixty-eight individuals at the peak of the spawning season. Seed (1976) found an average of 240 individuals on fronds of Laminaria saccharina collected from Puget Sound. A range of post-metamorphic size classes were often found in these populations. McBeth (1968) observed that the distribution of D. steinbergae on kelp fronds was markedly patchy; many colonies of Membranipora were free of the nudibranch while other colonies, of similar size, bore as many as twenty individuals.

McBeth (1968) confirmed that D. steinbergae feeds on zooids of Membranipora sp. He also found that other genera of encrusting bryozoans failed to elicit a feeding response. According to McBeth's (1968) description of feeding by adults, the oral lips are first applied to the frontal membrane of the zooid to form a seal and the radula subsequently rasps a hole in the frontal membrane. The contents of the zooecium are then sucked out by rhythmic dilations of the buccal pump. The ingestion process is assisted by the radula, which carries the more solid parts of the zooid into the buccal cavity.

Perron and Turner (1977) have reported the only previous study on the life history of a corambid nudibranch. They successfully reared Doridella obscura from the egg to the sexually mature adult. At a temperature of 25° C, the egg-to-egg generation time was only 26 days. The veligers of this species were planktotrophic and, following metamorphosis, the juveniles fed on epibenthic diatoms and organic debris for 5 days before they began feeding on zooids of Electra [Membranipora] crustulenta.

The life history of Doridella steinbergae was originally chosen as a research topic out of an interest in both nudibranchs and substratum selection by marine invertebrate larvae. This nudibranch seemed a suitable choice because it is easily obtained, has a telescoped life cycle

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during the benthic stage, and the adults exhibit a marked substrate preference (Kozloff, 1973). After laboratory rearing of the larvae was accomplished, and metamorphosis induced, specific questions were recognized which determined the direction of the subsequent research. The first of these dealt with the elucidation of the histological changes undergone by the planktotrophic veliger during the larval phase. The metamorphic process in *D. steinbergae* provided the opportunity to study the mechanism by which the anus and branchiae of corambids becomes situated below the rim of the notum. In addition, an attempt was made to examine the conversion of the larval gut of the planktotrophic veliger into the gut of the carnivorous adult.

Finally, the problem of juvenile feeding was investigated. Bryozoans, unlike sponges, do not lend themselves to micrograzing. It seemed that the necessity of penetrating the frontal membrane, and the removal of the zooid body from the zooecium, must present extraordinary mechanistic problems for juveniles measuring from 1/3 to 1/5 the length of the bryozoan zooecia.

MATERIALS AND METHODS

The life history of Doridella steinbergae was studied at Friday Harbor Laboratories, University of Washington, at Friday Harbor, Washington during the periods from June to September in 1976, and from May to August in 1977. Most of the nudibranchs were collected from fronds of the brown algae, Laminaria saccharina and Costaria costata which grew on the floating docks of the laboratory. Other specimens of D. steinbergae were obtained from beds of Nereocystis luetkeana, located at Parks Bay, Shaw Island and at Mar Vista, San Juan Island. At every location the nudibranch was found only on kelp fronds which bore epiphytic colonies of the bryozoan, Membranipora villosa.

Juveniles and adults were kept in the laboratory within culture bowls provided with pieces of algae encrusted with colonies of M. villosa. One to four animals were maintained in each bowl. The water was changed every day and, when necessary, the bryozoan was replenished with freshly collected colonies.

Egg masses which were to be used for embryological observations or for obtaining larvae for culture were removed from the adult bowls within 24 hours of oviposition. The spawn were carefully scraped from the substrate using a drawn-out glass pipette. These egg masses were kept in small jars and the water replaced every second day. One day prior to hatching of the larvae, flakes of cetyl alcohol were sprinkled on the water surface. This chemical prevented the hydrophobic shells of the veligers from becoming trapped in the surface tension (Hurst, 1967).

Two techniques were used for culturing the free-swimming larvae. The first of these, culture technique I, was employed during the summer of 1976. The larvae were cultured at a density of 1 to 4 larvae/ml. in glass beakers containing 500 mls. of sea water. The sea water was previously filtered through a Millipore prefilter (cat. no. AP 2004700). Two species of golden-brown algae, Monochrysis lutheri and Isochrysis galbana were added to the cultures as food for the veligers. The algae were cultured in Provasoli's E.S. Medium under conditions of continuous aeration and illumination. Although attempts were made to maintain axenic algal cultures, some bacterial contamination was unavoidable. According to culture technique I, the larvae were fed directly from the algal cultures. The algal concentration was quantified using a hemacytometer and the appropriate volume added to the culture medium to produce a concentration of 10^4 algal cells/ml.. In an attempt to control bacterial growth, the antibiotics, Penicillin G and Streptomycin sulfate (Sigma Chemical Company) were added to the larval cultures at a concentration of 0.06 mg/ml. and 0.05 mg/ml., respectively. Finally, flakes of cetyl alcohol were sprinkled on the water surface of each culture.

In culture technique I, the sea water, food, and antibiotics were renewed every second day. The method employed for transferring the larvae to fresh culture medium was that of Switzer-Dunlap and Hadfield (1977; related by Kempf, 1976). For this purpose, a sieve was constructed by replacing the bottom of a small plastic cup with Nitex cloth of a 57 µm pore size. This sieve was placed within a shallow dish and the larval culture was slowly poured into the sieve. As a result, the larvae were retained in a shallow layer of water within the sieve while most of the old culture water ran through the sieve and flowed over the sides of the shallow bowl. Freshly prefiltered sea water was then passed through the sieve to flush out most of the debris and old culture water surrounding the larvae. Using a Wild dissecting microscope, the larvae were then individually pipetted from the sieve into a beaker containing freshly prefiltered sea water, algae, and antibiotics.

Using technique I, the number of larvae which survived to metamorphic competence was very low - less than 1 percent of the original number. In an attempt to increase this yield, a modified procedure, culture technique II, was tried during the summer of 1977. Using technique II, survival of the larvae to the stage of competency was increased to approximately 80 percent.

According to technique II, the veligers were cultured in only 100 mls. of prefiltered sea water which was contained within small custard bowls. The initial larval concentration did not exceed 2 to 3 larvae/ml.. As in technique I, the larvae were fed Monochrysis lutheri and Isochrysis galbana at a concentration of 10^4 algal cells per ml. but the algal cells were cleaned prior to their introduction to the larval cultures. This was done by centrifuging subsamples of the algal cultures. The supernatant was discarded and replaced with freshly prefiltered sea water. Each subsample was centrifuged and resuspended in prefiltered sea water twice before the algal concentration was determined by hemacytometer and the appropriate volume of algal suspension added to the culture water. No antibiotics were used in technique II, and the larval cultures were changed every day rather than every second day. The same type of sieve was used to change the culture water, but it was employed in a different manner. Rather than pouring the culture through the sieve, the sieve was gently immersed into the culture and all but a shallow layer of culture water was removed by an aspirator placed inside the sieve. The culture bowl containing the larvae was then refilled with freshly prefiltered sea water and the procedure repeated. After removal of the sieve, the concentrated larvae were pipetted from the old culture bowl into one containing prefiltered sea water and the algal food. In contrast to the method of culture changing used in technique I, very few or no larvae were lost during the culture change procedure of technique II.

The embryological differentiation of the zygote, until the time of hatching, was followed by sequential observations of living embryos. Histological examination of these stages was not performed.

The development of the larvae, from hatching to metamorphosis, was monitored by daily observations of living veligers and by histological examination of specimens which were fixed at various stages of development. Photographic record was kept of the developmental stages undergone by the larvae. Larvae to be photographed were pipetted into a small drop of sea water on a microscope slide. To this was added several drops of Chlorobutanol (saturated solution in Millipore filtered sea water) and the slide was placed in the freezer compartment of a refrigerator (-20° C) for one minute. This procedure effectively relaxed the muscles of the veligers, thus preventing retraction of the cephalopedal mass into the larval shell (Bonar and Hadfield, 1974). However, since Chlorobutanol does not arrest ciliary activity, it was necessary to hold the larvae in a fixed position while taking measurements and photographs. For this purpose, small plasticine supports were placed on each corner of a coverslip which was then lowered over the wet mount. Using strips of filter paper, the excess fluid was absorbed from the preparation until the larvae were caught between the microscope slide and the coverslip. Photographs of live veligers, post-larvae, and juveniles were taken with Plus X film (Kodak) using a Wild M-20 compound microscope equipped with a camera. Photographs of adults were taken with a Pentax Spotmatic camera equipped with extension rings.

Preparation of speciments for 1 µm sections was performed according to the technique of Dunlap (1966) and Cloney and Forey (1968). Larvae were first relaxed in a solution of Chlorobutanol (1 part saturated solution Chlorobutanol:3 parts Millipore filtered sea water) at 0°C for 5 minutes. Following removal of the anaesthetizing solution, the larvae were flooded with primary fixative. This consisted of 2.5% glutaraldehyde (Ladd Chemical Company) in phosphate buffer (pH 7.6). The osmolality of the fixative was adjusted to 960 milliosmoles with 0.34 NaCl. The larval shells were decalcified according to the technique of Bonar and Hadfield (1974). Following 30 minutes in primary fixative, the larvae were transferred to a solution of 1 part primary fixative:1 part EDTA. Decalcification required 60 to 90 minutes to complete. The specimens were then rinsed in primary fixative followed by three, 5 minute rinses in 2.5% $NaHCO_3$ (pH 7.2). The larvae were post-fixed for 1 hour in equal parts of 4% OsO_4 (Ladd Chemical Company) and 2.5% $NaHCO_3$.

Specimens were dehydrated in a graded series of alcohols and embedded in Epon plastic (Shell Chemical Company).

For fixations of metamorphic stages, competent veligers were induced to metamorphose on small squares of algae encrusted with M. villosa. At specific time intervals after loss of the larval shell, the post-larvae were removed from the substrate by water jets applied by pipette. These specimens were then individually relaxed and prepared for histological examination according to the same technique outlined for the larvae (omitting the decalcification procedure). Fixations of metamorphic stages were made immediately after shell loss, and at 1/2, 2, 5, and 48 hours after shell loss. Juveniles of various ages and adults were also fixed for histological examination.

One micron sections of the fixed specimens were cut with glass knives on a Sorval MT-1 microtome. Sections were stained according to the technique of Richardson et al. (1960). Photographs of histological sections were made using Pan X film (Kodak) and a Zeiss Photomicroscope.

For experiments dealing with the induction of metamorphosis, and for observations of juvenile feeding, it was desired to obtain colonies of M. villosa which were encrusted on glass slides. For this purpose, plexiglass frames were contructed which held twenty, 51 x 75 mm. glass microscope slides. Since Crisp and Williams (1960) have shown that filming inert surfaces with algal extracts enhances settlement by bryozoan larvae, the microscope slides were treated in crude extracts of *Laminaria saccharina* for 24 hours. The slides were then placed in the frames and these were immersed into the ocean from the floating dock of Friday Harbor Laboratories on May 12, 1977. Colonies of *M. villosa* began appearing on these slides within 6 weeks.

RESULTS

A. OVIPOSITION AND EMBRYOGENESIS

Under laboratory conditions, adults lay one or two egg masses per day, and oviposition takes place mainly during the late evening and morning hours. The adults do not exhibit a substrate preference for oviposition. In the field and in the laboratory, adults lay egg masses on both the algae and the bryozoan; the laboratory-held animals also deposit spawn on the sides of glass bowls. A tendency was noted for egg masses to be deposited in aggregates.

The number of eggs per spawn ranged from 175 to over 2000 eggs. Although the egg masses are most commonly in the shape of an open circle, the two ends of the spawn are overlapped in larger egg masses or the egg ribbon is folded in secondary loops. Spawn were never observed in the shape of a spiral, as has been reported for Doridella obscura (Franz, 1967; Perron and Turner, 1977) and for Corambe pacifica (MacFarland and O'Donoghue, 1929).

The egg mass of D. steinbergae exhibits the characteristics of Hurst's type A opisthobranch egg mass. The primary egg cases, each containing one embryo, are embedded in a semi-flattened ribbon of jelly which is attached to the substrate along one edge. As the egg mass is extruded, the jelly is soft and sticky. However, within several hours after oviposition, this material becomes tough and elastic. Just prior to hatching, the jelly matrix appears to progressively deteriorate and is easily pulled apart. This degenerative change accompanies the hatching process regardless of the length of the embryonic period, which can be altered by manipulation of the ambient temperature.

The fertilized eggs of D. steinbergae are deposited in the primary oocyte stage. The ovum, which is 75 to 85 µm in diameter, undergoes two meiotic divisions and subsequently cleaves in a spiral pattern. The first two cleavages are holoblastic and almost equal. The third cleavage produces the first quartet of micromeres which are spirally positioned on the four macromeres. Subsequent cleavages produce a stereoblastula at approximately 48 hours after oviposition (at 12 to 15°C). The stereoblastula undergoes gastrulation by epiboly and at the completion of the third day, the foot and the velar rudiments have appeared. At this stage, the velar rudiment bears short, motile cilia. The foot rudiment is a broad rounded protuberance without an operculum at this stage of development.

On the fourth day, the shell gland, which previously spread over the posterior portion of the embryo, begins to secrete a thin, glistening shell.

During the fifth day, the beat of the velar cilia becomes metachronic. The effective stroke occurs to the left of an observer looking in the wave direction. This metachronal pattern is termed laeoplectic by Sleigh (1974). Shortly afterward, the embryo acquires the ability to simultaneously arrest the velar cilia. Previously, these cilia would often cease beating, but this occurred only in an erratic manner or in isolated groups. Meanwhile, the shell gland, or presumptive mantle, continues to advance anteriorly. Its migration is accompanied by continued shell secretion at its leading edge. Eventually, that portion of the mantle which is attached to the growing shell moves outward from the dorsal and lateral sides of the embryo. The shallow pocket of mantle epithelium which is left bridging the gap between the shell and the body proper is the mantle cavity. That portion of the shell gland which lies against the secreted shell is now termed the perivisceral membrane. A shallow space, the perivisceral cavity, soon appears between the perivisceral membrane and the differentiating viscera.

By the end of the fifth day, two distinct ridges are present close to the perimeter of the velar lobes. The velar ridge is formed by large cells bearing the preoral ciliary band. The cells of the subvelar ridge, which is located just below the velar ridge, bear the post-oral ciliary band. The food groove is the gulley between the two parallel ridges which circumscribe the velar margin.

Approximately ten hours after the ability to simultaneously arrest the cilia is acquired, the stoppages become accompanied by slight inward flexion of the velar ridge. This movement is indicative of the functional differentiation of the cephalopedal muscle complex. Later in embryogenesis, inward folding of the entire velar lobe will accompany ciliary arrest.

Between the fifth and sixth day, the formerly rounded foot develops a blunt point at its apex, and an operculum is evident on its posterior face. Long, stiff cilia project from both the apex and at various sites on the lateral sides of the foot. By the end of the sixth day all the visceral organs of the developing veliger, and the larval retractor muscle, are recognizable. Observations of live embryos indicate that the anus differentiates in a post-torsional position on the right, lateral side of the veliger. Full, 180° torsion, which would place the anus in a dorsal position, is never achieved in _D_. _steinbergae_.

Hatching occurs between 7 1/2 and 8 days after oviposition at a temperature of 12 to 15°C, and between 11 to 12 days at a temperature of 9 to 10°C. A summary of the major embryological events and the time of their appearance, at a temperature of 12 to 15°C, is presented in Table 1.

B. LARVAL DEVELOPMENT

Newly hatched veligers of D. steinbergae must undergo an obligatory period of planktotrophic development in order to attain metamorphic competence. The progressive changes in the external appearance of the veligers during the larval phase are shown in Figures 2 and 3. To facilitate the documentation of larval development, it is convenient to categorize the veligers into four stages. The onset of each stage is characterized by the appearance of readily recognizable morphological markers. This method of estimating maturity was found to be preferrable to age because, although morphogenesis proceeds according to a predictable pattern, the entire programme of events may require a variable length of time depending on such parameters as the water temperature and the availability of food. Veligers raised according to culture technique I required 35 to 37 days to reach metamorphic competence, while those raised according to culture technique II attained competence at 25 to 26 days after hatching.

Stage I begins when the veligers hatch from the egg mass. At this time, the larvae have a translucent digestive gland and a larval shell which consists of only one-half whorl (Fig. 2a, 2b). During stage I, the larval shell and the left digestive gland grow progressively (Fig. 4), and the latter structure becomes heavily pigmented (Fig. 2c, 2d, 2e, 2f, 3a).

Stage II veligers are identified by two features which develop concurrently: the paired eyespots and the retraction of the mantle from the aperture of the shell (Figs. 3b, 3c). Shell growth is arrested at stage II but the digestive gland continues to enlarge as larval development proceeds (Fig. 4). The first appearance of the propodial rudiment characterizes stage III veligers (Fig. 3d). The final stage, stage IV, is identified by the full development of the propodium (Fig. 3f). Although this stage is the most arbitrary in terms of morphological criteria, it can be confirmed if the veligers exhibit the ability to crawl. Stage IV veligers are competent to metamorphose if appropriately stimulated.

By histological examination of veligers fixed at each of these stages, the progressive development of eight major structures of the larvae will be presented. These are: the foot, the alimentary tract, the nervous system, the kidney complex, the larval heart, the gonad rudiment, the mantle fold, and the muscle systems.

1. Foot

Although the foot of gastropod veligers ultimately consists of both a proximal propodium and a distal metapodium, the stage I veliger of D. steinbergae possesses only the metapodial rudiment (Figs. 2a, 5a). This is a small, conical structure with a circular operculum attached to its dorsal face. At this stage, the periphery of the operculum extends well beyond the lateral edges of the foot. A pair of short, ciliated tracts extend from the mouth to midway down the lateral sides of the foot and a longer tract is located in a groove which runs down the midline of the ventral surface (Figs. 5a, 6a). In addition, isolated ciliary tufts are located at the apex and at paired locations on the sides of the foot. In life, the cilia of the tracts are motile and function to carry rejected particles away from the mouth. Each of the tufts is composed of a group of long, non-motile cilia (Fig. 6b) which adhere together and project beyond the rim of the operculum (Fig. 5d).

Two types of epidermal glandular elements are present in the metapodial rudiment of stage I veligers. A single pair of type 1, unicellular mucous glands are located on each side of the base of the foot (Fig. 6a), and a pair of multicellular glands which contain a purple-staining (Richardson's stain), reticulate material open into the pedal groove midway along its length (pedal groove glands) (Fig. 5a). The remainder of the foot epidermis is composed of simple cuboidal epithelium.

By stage II, the metapodium of the veliger has expanded to the extent that it occupies most of the operculum in live veligers. This growth is the result of proliferation and hypertrophy of the pedal epithelial cells (Fig. 6b). In addition, both the number and size of the cells composing the pedal groove glands has increased (Fig. 5b).

At stage III, a second type of unicellular mucous gland has appeared in the pedal epithelium (Fig. 6c), and the number of type 1 mucous cells has increased to three pairs. All of the latter are located on lateral portions of the foot. However, the most notable development which appears at the onset of this stage is the presence of numerous, undifferentiated cells within the proximal portion of the foot (Fig. 6c). The proliferation of these cells, which likely represent the prospective metapodial and propodial glands, is responsible for producing the initial swelling of the propodial rudiment.

Radical changes in the shape and tissues of the foot take place during stage III. By stage IV, the entire ventral surface of the foot is covered with a dense ciliation (Fig. 5c). Internally, the metapodial glands are large due to distension of the cells by their secretory product (Figs. 5c, 6c). The bulk of these glands occupies much of the propodial and proximal metapodial regions (Fig. 5c), and their ducts open onto the propodium. At stage IV, the foot is also characterized by the differentiation of two additional, multicellular pedal glands. The propodial glands are located proximal to the metapodials and a number of accessory metapodial glands are located within the distal portion of the metapodium

(Fig. 5c).

In summary, the stage IV veliger of D. steinbergae contains six distinct types of pedal gland cells. These are: the pedal groove glands, two types of unicellular mucous glands, the metapodial glands, the accessory metapodial glands, and the propodial glands.

2. Alimentary Tract

The organization of the larval alimentary tract can be seen in the photomicrographs of living veligers in Figures 2 and 3. The foregut consists of a tubular esophagus which extends posteriorly from the mouth. The midgut is composed of a stomach, a large left digestive gland, and a small right digestive gland. The esophagus and the lumen of the left digestive gland open into the ventral portion of the stomach. The hindgut, or intestine, leaves the dorsal extremity of the stomach, curves to the right, and then runs anteriorly to open into the right mantle cavity.

If offered suitable species of unicellular algae, the larvae of D. steinbergae will begin to feed immediately after hatching. At this stage, scattered yolk particles are still present in the tissues of the gut.

During stage I, the esophagus is composed of simple, cuboidal epithelium throughout its length (Figs. 7a, 7b). The cells bear motile cilia which are responsible for carrying food particles from the mouth to the stomach.

A single pair of unicellular gland cells is found in the epithelium of the distal end of the esophagus (Fig. 7a). The secretory product of these glands appears granular and stains purple with Richardson's stain. The prospective rudiment of the radular sac becomes apparent in stage II as a thickening of the ventral wall of the distal esophagus (Fig. 7c). At stage III, this portion of the esophagus has evaginated to form the rudiment of the radular sac (Fig. 7d). The radular sac has elaborated six pairs of teeth by stage IV (Fig. 7e). As the radular rudiment is formed, it becomes encircled by a number of developing muscle cells. These are the precursors of the odontophore musculature (Fig. 7e).

The oral lip glands, which become functional after metamorphosis, are present in rudimentary form in competent veligers. These glands contain small, spherical granules and are located above the distal end of the esophagus (Fig. 7f).

From the time of hatching, the stomach is clearly divided into ventral and dorsal chambers (Fig. 8a). The former portion receives the openings of the esophagus (Figs. 8a, 8b) and the left digestive gland (Fig. 8c). Cilia are present in the ventral chamber around both the esophageal-stomach junction and the entry of the left digestive gland. The remainder of the ventral stomach is lined internally with a layer of amorphous material which, in conformance with the terminology of Fretter and Montgomery (1968), will be called a cuticle (Figs. 8c, 8d). This material may be secreted by unicellular gland cells which are found only in non-ciliated regions of the ventral stomach (Fig. 8c). On the right side of the ventral stomach, opposite the entry of the left digestive gland, a number of rod-shaped bodies are embedded in the cuticle (Fig. 8d). These bodies are oriented perpendicular to the wall of the stomach and their distal ends extend to the surface of the cuticle. In live veligers, these rods are refractile.

The dorsal and ventral stomachs are demarcated by a slight thickening of the stomach wall which constricts the lumen (Fig. 2d). The epithelium of all but the upper wall of the dorsal stomach is densely ciliated. In fixed material, these cilia are uniformly oriented with their long axes perpendicular to the stomach wall (Fig. 8e). An intestinal groove runs down the midline of the anterior (upper) wall of the dorsal stomach to the origin of the intestine (Fig. 8e). The tract of cilia which lies within the intestinal groove is a continuation of the ciliated field in the ventral stomach. A translucent, rod-shaped structure was occasionally seen in the dorsal stomach, extending from the intestine to the ventral stomach (Fig. 8c). In living veligers, this rod is rotated by the cilia of the dorsal stomach, and turns in the same direction as the food bolus.

The intestine is a simple ciliated tube which terminates at the anus in the right mantle cavity (Figs. 8d, 13c).

The proximal portion of the esophagus, the stomach, and the intestine grow continuously up to stage IV. The only other change in these tissues is a progressive accumulation of intracellular, spherical granules of various sizes (Fig. 8b). These stain green with Richardson's, suggesting a lipid content.

Both a left and a right digestive gland (synonymous terms are left and right midgut diverticula) arise from the stomach wall of _D_. _steinbergae_ (Figs. 2d, 8c). The right gland is small, lacks a lumen, and remains unchanged throughout larval development. The cells of the left digestive gland are larger and more numerous than those of the right, and undergo considerable change during the larval period. In stage I larvae, the cells of the left digestive gland surround a spacious lumen which makes broad communication with that of the ventral stomach. The majority of these cells are cuboidal in shape and are sparsely ciliated (Fig. 9a). The only inclusions which are recognizable in one micron sections are large, round vesicles containing a granular material which stains a dense purple-blue (Fig. 9a). These inclusions can be found within the cells of the left digestive gland throughout larval development (Figs. 9b, 9c, 9d). In young veligers, a group of cells belonging to a second type are located in the posterior wall of the gland where it connects with the stomach. At hatching, these are large and yolky and contain large nuclei with prominent nucleoli (Fig. 9a). In photomicrographs of young larvae, these cells are apparent as a prominent bulge in the wall of the digestive gland (Fig. 2b), which does not become coloured with algal pigments after larval feeding has begun (Fig. 2c).

By stage II, the veligers have been feeding for a minimum of 10 days, and the pigments of the algal food confer a gold-green colour to the left digestive gland. The size of the gland has increased considerably by this stage due to an increase in its constituent number of cells (Figs. 4, 9b). In addition to the densely-staining, granular inclusions, some of the gland cells in stage II veligers contain large, spherical vacuoles which are filled with a reticulate or flocculent material (Fig. 9b). Various sized, spherical vesicles, containing a lipoid-like substance (stain green with Richardson's) also begin to appear in the digestive gland cells at this stage. The large, undifferentiated cells of the digestive gland are no longer present in stage II veligers.

By stage III, the size of the left digestive gland, as determined by the digestive gland index, is triple that of its post-hatch condition (Fig. 4). The cells of the gland have become large and pyramidal in shape, possibly to accomadate an increase in the number and size of both the flocculent vacuoles and the lipoid deposits (Fig. 9c). The enlargement of the gland cells results in partial occlusion of the lumen (Fig. 9c).

The left digestive gland of stage IV larvae resembles that of stage III, except the content of lipoid vesicles is further increased (Fig. 9d). In live veligers, the gland is a dark, gold-brown colour at this stage of development.

During the larval phase, growth of the left digestive gland is not strictly linear. When the digestive gland index is plotted as a function of veliger age, the resultant curve possesses three plateaus. The positions of each of these plateaus exhibit distinct correlation with the attainment of stages II, III, and IV, respectivelly (Fig. 4). The growth of the left digestive gland obliterates the perivisceral cavity on the left side of the veliger, but does not affect the relative position or orientation of the stomach (Fig. 14e).

3. Nervous System

The only ganglia present in stage I veligers of D. steinbergae are the cerebrals (Fig. 10a). The members of this pair are located on the dorso-lateral sides of the distal end of the esophagus, and a connecting cerebral commissure runs dorsally over the esophagus. Although small at this stage, each of the cerebrals is differentiated into an outer cortex, composed of nerve cell bodies, and an inner medulla, composed of nerve fibers. The statocysts are the only obvious sensory organs which are present in newly hatched veligers (Fig. 10b). Although pedal nerve cells may be present at this stage, they are not organized into distinct ganglia.

Considerable development of the nervous system is apparent in stage II veligers. The number of nerve cells within the cerebral ganglia has increased (Fig. 10c) and the pedal ganglia have become distinguishable (Fig. 10d). The pedals, which are not yet differentiated into cortex and medulla, are located on the ventro-lateral faces of the statocysts. A third pair of ganglia, the pleurals, have developed in a position dorsal to the statocysts and postero-dorsal to the cerebrals (Fig. 10e). These ganglia have both a medulla and cortex at stage II. The cerebral and pleural ganglia of each side are joined by a cerebro-pleural connective. The onset of stage II is characterized externally by the development of the paired eyespots. These differentiate in close association with the cerebral ganglia, in the region just behind the velar lobes. The pigmented cells of the eye are arranged in the form of a cup which is oriented with the concave side facing antero-laterally. A small, spherical lens is produced within the cavity of the cup and this becomes covered with a transparent cornea. As the eyes form, the adjacent portions of the cephalic epithelium invaginate and these cells ultimately become the paired optic ganglia (Fig. 10e).

The cerebral ganglia, particularly the medullary component, continue to enlarge during stage II (Fig. 11a). However, the most notable changes which have occurred by stage III are the marked enlargement of the pedal ganglia (Fig. 11b) and the differentiation of the buccal ganglia (Fig. 11c). Coincident with the growth of the pedal ganglia, each becomes organized into a medulla and cortex. In addition, pedal and cerebro-pedal connectives become apparent.

The differentiation of the buccal ganglia occurs as the rudiment of the radular sac evaginates from the ventral wall of the esophagus. The buccal ganglia, which possess both cortex and medulla at this stage, lie lateral to the radular rudiment, and the buccal connective passes between this rudiment and the esophagus (Fig. 11c). Slender cerebro-buccal connectives are also present.

No additional ganglia differentiate between stages III and IV. However, the cerebral (Fig. 11d), the pedal (Fig. 11e), and the buccal (Fig. 11f) ganglia continue to enlarge, mainly as a result of an increase in nerve fibers within the medulla.

4. Larval Kidney Complex

The components of the larval kidney complex will be defined as those cells which are secretory in appearance, and which open into the right mantle cavity, adjacent to the anus. Four of these cell types are present in stage I larvae. The most conspicuous is a large cell with a vacuolate cytoplasm which is organized around a large central vacuole (Fig. 12a). The nucleus and nucleolus of this larval secretory vesicle (terminology from Thompson, 1958) are very large. The type a larval kidney cell is much smaller than the larval kidney vesicle, but it also possesses a vacuolate cytoplasm (Fig. 12a). The type b kidney cell is filled with a reticulate product (Fig. 12a), while the inclusions of the type c cell are small, spherical granules. The ducts of the kidney vesicle and the type a and b cells are located dorsal to the anus (Figs. 12b, 12c). No duct was apparent in the type c cell. The size of the vacuoles in the type a cell and the central vacuole of the kidney vesicle enlarge throughout larval development (Figs. 12c, 12d). During stage III, a final cell type, the type d cells, differentiate. The cytoplasm of these cells is finely divided into small locules (Fig. 12d). At stage IV, the rudiment of the adult kidney is present. This is an oblong structure, consisting of vacuolate cells, which is located on the dorsal face of the kidney vesicle

(Fig. 12e).

5. Larval Heart

Regular pulsations of the tissue overlying the dorsal esophagus become apparent at the onset of stage II. These pulsations were seen only in unanaesthetized veligers. The pulsations were presumed to represent the differentiation of the larval heart. However, in one micrometer sections, no tissue specialization was recognizable in this region. The rudiment of the adult heart does not appear in the larval stage of D. steinbergae.

6. Gonadal Rudiment

In newly hatched veligers, a large granule of dense material, which is encircled by squamous cells, is located immediately ventral to the terminal intestine (Fig. 13a). By stage II, this granule (which is presumed to be yolk) is no longer present. In its place, 7 to 9 large cells have differentiated. The nuclei of these cells are large and their nucleoli are prominent; the cytoplasm stains lightly and contains numerous, small granules (Fig. 12b, 12c). The cells progressively enlarge as the larvae develop. As a result of their growth, and the concurrent enlargement of the left digestive gland, the cells become situated on the right ventro-lateral face of the digestive gland, just ventral to the anus (Fig. 13d). The histological appearance of these cells, and the position they migrate to at metamorphosis, suggests that these are the primary germ cells of the gonad.

7. Mantle Fold

At hatching, the shell of D. steinbergae consists of only one-half whorl. As a result, if serial, transverse sections are cut through the antero-posterior axis of stage I larvae, the shell and perivisceral membrane appear bilaterally symmetrical in all but the most ventral sections (Figs. 10a, 10b). However, subsequent growth of the larval shell is sinistral and therefore asymmetric. As a result, the mantle cavity on the left side becomes obliterated by the shell whorl (Fig. 14e). At stage I, the mantle cavity is very shallow and the mantle cells which are attached to the rim of the shell are actively engaged in shell deposition. The cells of the mantle fold are few in number and cuboidal in shape (Fig. 10a).

At stage II, the mantle undergoes a series of changes. As shown in the photomicrographs of living veligers (Figs. 3b, 3c), the mantle fold detaches from the shell rim and retracts towards the posterior end of the larva. This event marks the end of shell growth (Fig. 4). As a result of mantle retraction, there is a positional change of the distal periphery of the fold, and the depth of the mantle cavity is markedly increased (Fig. 14a). Histological examination of larvae during the process of mantle retraction indicates that three processes are involved. Firstly, during the latter part of stage I, the cells of the inner epithelium of the mantle fold proliferate slightly. At the onset of mantle retraction, these cells undergo a change in shape from cuboidal to squamous (Fig. 14b). Coincident with the cell shape change, slender muscle processes, which insert on the mantle fold or the associated cells of the kidney complex, and on either the perivisceral membrane (Figs. 15a, 15c) or the visceral organs (Fig. 15b), begin to contract. This activity provides the force to pull the mantle epithelium posteriorly and the change in cell shape provides the epithelium with the extra surface area required to line the deepened mantle cavity.

Unlike the growth of the larval shell, which is sinistral, the body of the larvae twists dextrally as it grows. This condition is termed hyperstrophy. The dextral pattern of visceral flexure can be demonstrated by following the orientation of the esophagus (which is common to both the cephalopedal mass and the visceral mass) through serial, transverse sections. Toward its proximal end, the dorso-ventral axis of the esophagus and the rudiment of the radular sac can be seen to twist clockwise as they conform to the dorso-ventral axis of the visceral mass. Dextral visceral flexure and mantle retraction result in a change in the position of the anus and kidney complex, relative to the adjacent portion of the esophagus. Prior to mantle retraction, the anus and its associated larval kidney structures are located adjacent to the distal esophagus, on the right side of this tube. However, mantle retraction draws the anus to a position adjacent to the proximal end of the esophagus. Since this end of the esophagus is twisted clockwise, the retracted anus and kidney complex lies ventral to the proximal end of the esophagus, rather than lateral to it. This reorientation will have implications at metamorphosis.

At stage III, further change in the mantle tissue is apparent. Although the cells which line the floor of the mantle cavity and those of the outer mantle fold epithelium remain squamous, the cells of the inner mantle fold epithelium begin to proliferate and become cuboidal in shape (Figs. 14c, 14d). This process continues until, by stage IV, the inner epithelium consists of numerous columnar cells. Some of these have differentiated into unicellular gland cells (Fig. 14f). A ciliated tract is elaborated by the cells which run along the distal edge of the fold (Fig. 14f).

8. Muscle Systems

Three muscle systems are present in the veliger of D. steinbergae. These are: the larval retractor muscle, the subepidermal cephalopedal muscles, and the mantle retractor muscles.

As mentioned previously, the fibers of the mantle retractor muscles extend across the perivisceral cavity prior to and during mantle retraction. Three types of connections were found. These are: between the perivisceral membrane and the mantle fold (Fig. 15a), between the perivisceral membrane and the kidney complex (Fig. 15c), and between the intestine and the mantle fold (Fig. 15b).

The larval retractor muscle is slender at stage I (Fig. 16a), but undergoes progressive hypertrophy during larval development (Fig. 16e). It originates on the inner wall of the shell, to the left of the mid-dorsal axis, and extends anteriorly along the left, dorso-lateral side of the esophagus (Figs. 16a, 16c). Distally, the muscle bundle divides into three main branches. Two of these insert on the epithelia of the paired velar lobes (Fig. 16b), while the third extends to the pedal region (Figs. 16a, 16e). In stage IV veligers, fibers of this latter branch insert on the inner epithelium of the propodium, on the pedal epithelium which is attached to the operculum (Fig. 16e), and on that portion of the periivisceral membrane which lines the ventral aperture of the larval shell (Fig. 16c). The epithelium of the latter insertion site is confluent with the mantle fold surrounding the anus, and with the pedal epithelium which is attached to the operculum. Beginning at stage III, the trunk of the retractor muscle establishes connections with the posterior end of both the stomach-intestine, and the left digestive gland (Fig. 16d).

Since the contraction of the larval retractor muscle pulls the body of the larva inside the shell, ~~it~~ normally does not become functional until after mantle retraction (stage II). Prior to this event, the mantle cavity is too shallow to accomodate the retracted larval body. However, if the veliger is exposed to noxious chemicals or severe mechanical disturbance, it will contract violently, causing a premature disengagement of the mantle tissue from the rim of the shell. The mantle retractor muscle also plays a major role in effecting the reorganization of the larval body during metamorphosis. Its role during this event will be discussed in a subsequent section.

The cephalopedal muscles consist of slender muscle fibers which run beneath the epithelium of both the foot and the velum. Although the velar component of this musculature is present at hatching, the pedal component does not become evident until stage III.

9. Behavior of the Veliger

The behavior of D. steinbergae veligers was not studied in detail. However, records of behavior, as it was observed during the culture of the larvae, were kept.

During the majority of the developmental period, until full development of the propodium, larval motility is completely dependent on the activity of the preoral ciliated band of the velum. Due to the organization of the larval body, the velum of swimming veligers faces upward while the visceral mass and shell are suspended below. As a result, increased activity of the preoral cilia automatically produces upward swimming. However, by adjusting the relative positions of the velar lobes, larvae are also able to swim horizontally and to turn. These movements of the velar lobes appear to be effected by the subepidermal muscle fibers. Since the density of the veligers is greater than that of sea water, the larvae sink when the beat of the preoral cilia is arrested.

Immediately after hatching, the veligers exhibit strong upward swimming. This behavior occurred regardless of the presence of light. However, within 24 hours and continuing throughout the period of larval culture, most of the veligers (approximately 70 per cent) were located close to the bottom of the culture bowls while the remainder were distributed throughout the water column. The tendency for the larvae to concentrate near the bottom became more apparent during the last half of their development. The larvae never exhibited a pronounced or predictable reaction to a directional light source.

Although full development of the propodium provided the larvae with the physical ability to crawl, this behavior was infrequent. However, several observations suggest that water turbulence may enhance the expression of crawling activity. If cultures of competent larvae were gently removed from the sea table and observed, the veligers were either swimming sluggishly over the bottom or lying on their sides. However, when these cultures were examined immediately after culture changing, a procedure which caused considerable agitation of the culture water, several veligers were usually observed crawling. This phenomenon was also observed if the culture water surrounding competent veligers was forcibly drawn in and out of a pipette.

C. INDUCTION OF METAMORPHOSIS

Although several hundred, competent *D. steinbergae* veligers were raised during this study, some for up to 50 days, only one individual was seen to metamorphose on the bottom of its culture bowl. Observations of both live and sectioned veligers, at various ages past the onset of stage IV, indicate that further development is arrested once competence to metamorphose is attained.

In the areas around San Juan Island, Washington, *D. steinbergae* was found only on algal fronds which bore epiphytic colonies of *Membranipora villosa*. The nudibranch populations on these fronds usually included a number of individuals which, judging from their size (less than 0.5 mm.), had only recently metamorphosed (Fig. 17). Since the small size of these juveniles, and the nature of their habitat (suspended fronds of kelp) would argue against the possibility of immigration from elsewhere, it appeared that active recruitment was occurring on kelp encrusted with *M. villosa*.

As a result of these observations, the inductive capacity of both *Laminaria saccharina* and *M. villosa* was tested using laboratory reared veligers of *D. steinbergae*. Larvae were offered one of four types of substrates: *M. villosa* encrusted on *L. saccharina*, *L. saccharina* alone, *M. villosa* encrusted on a glass slide, and a glass slide subjected to the same conditions as the former slide, but without colonies of M. villosa. Fifteen competent veligers were introduced into each of the experimental bowls. Two trials of this experiment were performed, one using 28 day old veligers, the other using 31 day old veligers. In the second trial, the experiment involving the glass slide alone was omitted. The results of these experiments are presented in Table 2. They indicate that M. villosa induces metamorphosis of competent D. steinbergae veligers. L. saccharina alone is not effective, nor is it necessary for induction by M. villosa.

Since D. steinbergae veligers exhibit the ability to postpone metamorphosis in the absence of M. villosa, experiments were performed to determine if extended periods of delayed metamorphosis affected the response of veligers to M. villosa. Each test involved 15 larvae, and 5 age classes were tested. These were: 24, 28, 31, 35, and 41 days after hatching. For purposes of comparison, larval response was quantified by determining the length of the latency period and the total number of metamorphoses for each age class. The number of metamorphosed individuals in each test bowl was recorded at approximately one hour intervals. Observations were discontinued at 15 hours after introduction of M. villosa.

Although only one trial was performed for each age group, the results (Fig. 18) indicate that larvae between the ages of 28 and 38 days old are equally responsive to

M. villosa. In each of these age classes, the majority of metamorphosed nudibranchs appeared during the first third of the observation period. Since most of the 24 day old larvae had not achieved maximum foot development, their behavior in the presence of M. villosa confirms the morphological indication that these veligers, with the exception of one, were not yet competent to metamorphose. Although several of the 41 day old veligers retained the capacity to undergo normal metamorphosis, the overall response of this age class was poor relative to the younger stages of competent veligers.

While performing the various induction experiments, and during inductions for the purpose of fixing metamorphic stages, numerous larvae were observed in the act of metamorphosis. In these cases, the actual site of metamorphosis on the induction substrate was recorded. Analysis of these records indicates that the veligers of D. steinbergae exhibit a distinct preference for metamorphosing on the surface of the algae (or the glass slide), with their anterior end in contact with the undifferentiated periphery of a colony of M. villosa (Fig. 19). Superimposed on this preference for the periphery of the bryozoan colony, the veligers seemed to exhibit a secondary preference for small indentations in the border of the colonies. These indentations were in the form of small imperfections in the expanding border of differentiating zooids, or notches produced at the merging margins of two confluent colonies. To test the validity of this observation, four pieces of *L. saccharina*, each bearing colonies of *M. villosa* exhibiting various forms of surface indentations, were selected. Each of these substrates was placed in a bowl into which 10 competent veligers were added. In Figure 20, maps of each of these substrates are drawn, and the sites at which larvae metamorphosed are indicated. Of a total number of 24 metamorphoses, 16 occurred at the periphery, and of these, 9 were within notches on the colony borders.

As a result of the preceding observations, experiments were designed to determine if the presence of the undifferentiated zooids of *M. villosa* colonies were actually required for metamorphic induction. Four experimental bowls were set up. Bowl one contained intact *M. villosa* colonies on *L. saccharina*. Bowl two contained colonies in which all the differentiated zooids were cut away, and bowl three contained colonies in which the undifferentiated zooids were cut away. In the fourth bowl, larvae were offered both types of experimentally altered *M. villosa* colonies. An attempt was made to keep the surface area of the bryozoan (composed of either differentiated or undifferentiated zooids) equivalent in each of the bowls. Fifteen larvae were added to each bowl and the number of metamorphosed juveniles was recorded after 48 hours. The results are summarized in

Table 3.

Relative to the control group (bowl one), low numbers of larvae metamorphosed in bowls two, three, and four. This may have been caused by the extensive areas of damaged bryozoan tissue which resulted from the experimental manipulations. Although this experiment should have been repeated, a sufficient number of larvae within the ages of 28 to 38 days was not available. Nevertheless, the results indicate that peripheral zooids are not necessary for induction, as 2 larvae metamorphosed in bowl three. However, since all of the 4 juveniles in bowl four metamorphosed on the peripheral zooids (three of these occurred at the bryozoan - algae junction), at least some support is given to the hypothesis that the periphery of M. villosa colonies is more attractive to competent veligers than the center of the colony. Possible reasons for this phenomenon will be given in the discussion.

Metamorphosis by D. steinbergae veligers is induced by a highly specific type of substrate. This suggested that the inducing factor was a unique chemical component of M. villosa. If so, sea water conditioned with M. villosa might retain this active entity. Even if this was the case, the ability of larvae to respond to conditioned sea water would depend on their ability to sense the inducing factor under artificial conditions. To investigate this possibility, colonies of *M. villosa*, encrusted on glass slides, were placed in a small bowl of sea water for a period of 48 hours. At the end of this period, the glass slides bearing the bryozoan colonies were removed and 15 larvae (31 days old) were placed in the conditioned sea water. Two larvae metamorphosed in this bowl.

D. MORPHOLOGICAL CHANGES DURING METAMORPHOSIS

Transformation of the pelagic, planktotrophic larva into the benthic, carnivorous juvenile occurs during the process of metamorphosis. Metamorphosis of _D. steinbergae_ was found to affect almost every component of the larval body. In order to describe the various changes which are triggered at this time, it is convenient to separate the events into two categories. The first of these will deal with the reorganization of the body plan. The second will deal with histological changes within the larval organs and the differentiation of adult structures. This categorization does not reflect a chronological order for the metamorphic events. Although the loss, resorption, or transformation of the various larval components and the differentiation of adult structures require varying lengths of time to complete, all the events appear to be initiated at the time of metamorphic induction and take place concurrently.

1. Reorganization of the Body Plan

In the presence of _M. villosa_, competent veligers crawl actively over the surface of the bryozoan and the substrate it encrusts. Cessation of crawling and the onset of shell pivoting behavior precedes the onset of metamorphosis. Shell pivoting involves a series of anteriorly directed rotations of the shell relative to the foot (Fig. 22c). During these movements, the foot remains in contact with the substratum along the entire length of its crawling surface. The duration of the period of shell pivoting varies from 5 minutes to several hours. The subsequent events of metamorphosis are documented in the series of photomicrographs in Figure 21. The first morphological sign of the onset of metamorphosis is the detachment of the larval retractor muscle from the posterior end of the larval shell, and the withdrawal of the large digestive gland from the shell whorl (Fig. 21b). Soon after, the ciliated cells of the velum begin to dissociate from the surrounding epidermis and, due to the active beating of their cilia, these discarded cell masses swim away from the metamorphosing veliger (Fig. 21c). The visceral mass of the veliger eventually pulls completely out of the larval shell (Figs. 21d, 21e, 21f) and the operculum is shed from the dorsal surface of the foot (Fig. 21b). Throughout the process of shell loss, the visceral mass continues to pivot in an anterior direction. As the larval shell is lost, the spherical larval kidney vesicle, and the type a larval kidney cells become dissociated from their surrounding tissues and are shed (Fig. 21c). Although the loss of the larval shell, operculum, velar cells, and larval kidney cells all take place during the first part of metamorphosis, they do not occur according to a fixed pattern in every veliger.

Immediately after shell loss, the metamorphosing larva has a prominent dorsal hump in which both the pigmented digestive gland and the stomach can be seen (Figs. 22d, 33a, 21f). For a period lasting about 5 to 10 minutes after shell loss, the body of the post-larva undergoes slight, repetitive contractions along a dorso-ventral axis running from the foot through the dorsal hump. In lateral view, the profile of this dorsal hump becomes gradually confluent with the anterior cephalic region and the dorsal part of the metapodium (Fig. series: 22d, 22e, 22f, 22g; Fig. series: 23a, 23c, 23d, 23e). This process is possible due to the previous removal of both the operculum and the larval shell. By 2 to 5 hours post-shell loss, the body has flattened considerably along its dorso-ventral axis, and the deep crevice which was formerly present between the posterior edge of the dorsal hump, and the upper surface of the metapodium has been almost obliterated (Fig. 22f). The result is a considerable broadening of the connection between the dorsal hump and the foot. Within the subsequent 48 hours, the lateral and posterior portions of the foot become overlapped by the peripheral rim of the notum (Figs. 22g, 23e). Thus, the dorid design of the animal becomes apparent.

In addition to the changes in body shape, examination of sectioned animals, which were fixed at various periods after shell loss, reveal that considerable changes in the internal organization of the larval body take place at metamorphosis.

Prior to metamorphosis, the large, left digestive gland and the stomach are located ventral to the antero-posterior axis of the larva, within the oldest part of the shell (Figs. 22a, 22b). The larval retractor muscle originates at the posterior end of the shell, immediately behind the stomach and digestive gland (Fig. 22b). The base of this muscle is connected to the dorsal stomach and proximal regions of the intestine, and to the posterior end of the digestive gland. However, immediately after shell loss, the trunk of the larval retractor muscle is located anterior to these visceral organs in a position dorsal to the left eyespot (Figs. 22d, 23a). The proximal portion of the muscle extends basally down the left side of the post-larva (Fig. 31a), and the distal fibers insert on the epithelia of the sole of the foot and the back of the metapodium (formerly occupied by the operculum) (Fig. 34a). In addition, the stomach and digestive gland have rotated anteriorly to a position which would be dorsal to the antero-posterior axis of the competent larva. As a result of this rotation, the posterior ends of both these organs become directed anteriorly (Figs. 22d, 34b).

Sections through animals immediately after shell loss reveal that, during their anterior rotation, the digestive gland comes to occupy a medial position with the stomach located on its dorso-lateral surface (Figs. 24c, 23b, 25b). By 48 hours after shell loss, the stomach is located mid-dorsally over the digestive gland (Figs. 23e, 24e).

The anus, which was located adjacent to the right, ventro-lateral face of the ventral stomach in the competent veliger (Figs. 22a, 24a), is located posterior to the repositioned stomach and digestive gland in the post-larva (Figs. 24b, 23c).

The most credible explanation for these positional changes is contingent upon the activity of the larval retractor muscle and the removal of the larval shell and operculum during metamorphosis. The shell pivoting behavior which was observed prior to, and during shell loss, is likely caused by repetitive contractions of the retractor muscle. These contractions may facilitate the detachment of the muscle trunk, along with the associated portion of the perivisceral membrane, from the inner wall of the larval shell. Since the foot appears to be firmly attached to the substrate at this time (to be discussed below), the continued contraction of this muscle pulls its trunk to an antero-dorsal position.

The posterior ends of both the dorsal stomach, and the left digestive gland, are pulled forward with the trunk of the retractor muscle due to the muscle connections which exist between these structures. As a result, their anterior regions are pulled away from the whorl of the larval shell and are subsequently rotated to a posterior position.

The removal of the larval shell and operculum, and the contraction of the unilaterally positioned retractor muscle, are responsible for eliminating dextral visceral flexure and altering the position of the stomach relative to the digestive gland. When the shell and operculum are discarded, the body of the animal is freed from their physical restriction. Consequently, the contraction of the retractor muscle can rotate the digestive gland anteriorly and simultaneously brings this organ to a more medial position, relative to the cephalic area. During the process, the dorso-ventral axis of the digestive gland is forced to conform with that of the cephlopedal mass. Thus, visceral flexure is undone.

The contraction of the larval retractor down the left side of the metamorphosing larva pulls the dorsal stomach to the left as it is brought forward. This action helps bring the stomach dorsal, rather than lateral, to the digestive gland (Figs. 22d, 22e, 24c). The process is completed by subsequent growth of the digestive gland beneath the stomach (Fig. 24e).

The posterior relocation of the anus is an indirect result of anterior rotation of the visceral mass, and the simultaneous elimination of visceral flexure. This process is shown in Figure 24. The anus becomes posterior because the visceral organs are rotated anteriorly (Fig. 24b). As this rotation occurs, the organs and portions of organs which were located below the distal esophagus in the competent veliger, swing anti-clockwise (as viewed anteriorly) as the dorso-ventral axis of the visceral mass is forced to conform to that of the cephalopedal mass. The organs affected include the gonadal rudiment, the proximal end of the esophagus, the ventral stomach, the ventral extremity of the digestive gland, and the anus and kidney components. The anti-clockwise shift of these structures is aided by contraction of those muscle fibers of the pedal branch which insert on the mantle tissue which lies ventral to the anus. As a result of these concurrent processes, the anus of the post-larva is located not only posteriorly, but also mid-sagittaly (Figs. 23c, 24d, 24e).

The contractions of the post-larval body, which occur for a brief period after shell loss, may be due to the continued contraction of the larval retractor muscle. The axis of these contractions, as seen in lateral view, coincides with the long axis of this muscle in specimens fixed immediately after shell loss. At this time, the activity of this muscle likely functions to complete the positional changes of the organs, and also to force incorporation of the visceral mass into the foot. The resultant broadening of the connection between the foot and visceral mass progressively increases the distance between the anus and the esophagus (Fig. 23).

The final, major change in body organization is the eversion of the mantle fold and the anterior displacement of the base of this fold. In the veliger, the base of the mantle fold is confluent with that portion of the perivisceral membrane to which the trunk of the retractor muscle is attached. When the muscle, and its associated perivisceral membrane, detach from the shell and contract, the base of the mantle fold is pulled anteriorly. As the larval body is squeezed from the shell, the narrow aperture of the shell pushes the free edge of the mantle fold posteriorly over the anteriorly shifted base of the fold. The result is a reflection of the entire fold of mantle epithelium.

As a result of mantle reflection, the layer of mantle epithelium composed of columnar cells interspersed with unicellular gland cells, faces externally (Fig. 23a). In the competent veliger, this layer forms the lining of the mantle cavity, which is no longer present after reflection of the mantle fold during metamorphosis. Immediately after shell loss, the dorsal body wall of the posterior half of the post-larva is composed of three epithelial layers. Proceding from the outermost layer, these are: the columnar layer of mantle fold epithelium, the squamous layer of mantle fold epithelium, and the squamous epithelim of the perivisceral membrane (Fig. 23b). The columnar layer of the mantle fold becomes the definitive dorsl epidermis, while the two innermost layers are no longer recognizable at 5 hours post-shell loss.

In the veliger stage, the mantle fold adjacent to the anus and adult kidney rudiment is short; its contribution to the sheet of reflected mantle fold is minimal. However, it is signifigant that the mantle tissue which surrounds the anus does not become everted. If this occurred, the adult kidney rudiment would be located on the left side of the anus after posterior relocation of this complex. This is not the case (Fig. 33c). Figure 24 illustrates how those portions of the mantle fold and perivisceral membrane, which are associated with the anal region, are displaced during mantle fold eversion. The floor of the mantle cavity, which runs from the trunk of the retractor muscle to the region adjacent to the adult kidney rudiment, is pulled anteriorly. Consequently, the mantle fold epithelium is connected to the cephalic and pedal regions of the body from the displaced trunk of the larval retractor muscle, along the right side, to the anterior edge of the anus. The free edge of the mantle fold, which has everted by this time, begins at the anterior edge of the anus and extends past the anal aperture and around the left side of the visceral mass. Therefore, the anal aperture is located beneath the free edge of the reflected mantle fold (Figs. 23c, 24b, 24c).

Eventually, the cells along the free edge of the mantle fold fuse with the lateral epidermis of the foot on the left side. This fusion process is not necessary on the right side as this portion of the epithelium has been continuous with the cephalic and pedal epithelia throughout the metamorphic process (Fig. 23b). At the terminal end of the animal, the cells at the free edge of the mantle fold fuse with those lining the dorsal wall of the anal aperture (Figs. 23d, 24d). This is the last area of fusion; it is completed approximately 5 hours after shell loss. As a result of this process, the visceral mass becomes completely enclosed by the mantle epithelium and the anus is located below the everted mantle tissue.

Proliferation of the mantle cells eventually produces a dorsal sheet of tissue which overlaps the anus and the posterior and lateral sides of the foot. This is the definitive notum of the benthic stage (Fig. 23e).

2. Histological Changes of the Larval Organs and the Differentiation of Adult Structures

Although some of the larval structures, such as the shell, operculum, velar cells, and kidney vesicle are simply shed at metamorphosis, other components of the larval body are retained. In these cases, the larval structure may be almost unaltered, or it may provide only a framework on which metamorphic modifications are superimposed. In addition to these transformation processes, uniquely adult structures are induced to differentiate. In some cases, these structures are recognizable as partially formed rudiments in the later stages of larval development. Examples of this are the rudiments of the adult kidney, gonad, and radular sac. Other adult structures, such as the salivary glands and the rhinophores, do not become distinguishable until 1 and 7 days, respectively, after shell loss.

a. Alimentary Tract

In _D_. _steinbergae_, the organs of the larval alimentary tract are retained at metamorphosis but undergo varying degrees of modification. Of the various organs comprising this system, the structure of the stomach undergoes the most dramatic alteration. Beginning immediately after shell loss, the cells of the dorsal stomach are completely eliminated. These cells are readily recognizable by the dense concentration of basal bodies at their apical ends. Although these structures are not individually resolvable in one micron sections, they appear as a heavily stained, granular lining located beneath the apical membrane (Fig. 25a). The majority of the dorsal stomach is still intact at the time of shell loss, but some of the cells which lie adjacent to the constriction between the dorsal and ventral stomach can be seen in the process of detaching (Fig. 25a). Dorsal stomach cells can also be seen in the lumina of both the ventral stomach and the left digestive gland (Fig. 25b).

At 30 minutes after shell loss, only the narrow, terminal half of the dorsal stomach remains intact (Fig. 25c), and large numbers of the dissociated cells are present within the ventral stomach and the digestive gland. By 2 to 5 hours after shell loss, the dorsal stomach has been completely disassembled. As a result, the proximal end of the intestine now connects directly to the sac-like ventral stomach (Fig. 26a).

Metamorphic changes also occur in the tissues of the ventral stomach. By 48 hours after shell loss, neither the cuticle nor the refractile rods are present in this organ (Fig. 26b). It is not known whether this situation has resulted from a complete sloughing of the cuticle-coated cells, or merely from a removal of the cuticle from the apical ends of these cells. However, since all the remaining cells of the ventral stomach are ciliated, and because the size of the ventral stomach is much reduced compared to that of the veliger stage, it would appear that the cuticle-coated cells have been discarded.

Morphogenetic changes in the buccal region of the alimentary tract become evident soon after shell loss. These changes anticipate commencement of the benthic feeding mechanism. The first development is the loss of the esophageal gland cells. These appear to slough into the lumen of the esophagus and are then passed to the stomach. In cross-sections of post-larvae at 5 hours after shell loss, the lumen of the esophagus is laterally compressed, and the epithelium surrounding the lumen is thickened (Fig. 27a). At 48 hours after shell loss, the slit-like appearance of the esophageal lumen is greatly pronounced. The epithelial cells have undergone further proliferation and hypertrophy, and the lumen is lined with a cuticle-like material (Fig. 27b). This region is now recognizable as the buccal pump. Figure 27c, shows the appearance of the buccal pump in a 10 day old juvenile. The walls of this organ are heavily muscularized.

In competent larvae, the cells of the prospective odontophore musculature encircle both the radular rudiment and the adjacent region of the esophagus. Although the muscle elements within these cells are poorly developed prior to metamorphosis, considerable development of this tissue occurs during the first 3 days after shell loss. Movements of the radula can be detected in 2 to 3 day old juveniles, thus indicating that the odontophore musculature has achieved a functional state.

Two types of glands are associated with the buccal mass in the benthic stage of D. steinbergae: the oral lip glands and the salivary glands. The former are present in rudimentary form in the competent veliger and can be seen in histological sections of early metamorphic stages (Fig. 28a). These gland cells enlarge progressively during the post-larval and juvenile stages. They eventually form two glandular masses, one on each side of the oral region, and give rise to numerous ducts which open through the peri-oral epidermis (Fig. 28b). This peri-oral region becomes elaborated into fleshy oral lips, which become recognizable between 48 hours and 3 days after shell loss (Fig. 34d). Oral tentacles eventually develop from the external, lateral surfaces of the oral lips (Fig. 28b).

The salivary glands originate as a pair of outpocketings from the lateral walls of the esophagus, just posterior to the developing buccal pump (Fig. 29a). At 48 hours after shell loss, small amounts of the characteristic secretory product of the salivary glands becomes evident within the cells which line these evaginations (Fig. 29a). Due to a rapid increase in both the number of cells, and the amount of secretory product in these glands, their size is equivalent to that of the radular sac in 3 day old juveniles (Fig. 29b). In juveniles and adults, the salivary glands are large and sausage-shaped. They completely encircle the esophagus and radular sac in the region immediately behind the swelling of the buccal pump (Fig. 29c).

During the period following shell loss, the stomach and digestive gland contain both algal cells and dissociated larval cells (Figs. 23b, 23c, 23d, 30a, 30b). Some of the cells are dorsal stomach cells, while others, which stain purple with Richardsons, are likely the esophageal gland cells. In some, but not all post-larvae, which were fixed before complete dissociation of the dorsal stomach, the lumina of the stomach and digestive gland contain a large number of larval cells (Fig. 34b). The most probable origin of these cells is the velar lobes. Although the majority of these cells, particularly those of the pre-oral ciliated band, dissociate and swim away from the metamorphosing veliger (Fig. 21c), it is likely that some of the velar cells may become caught in the ciliary current of the esophagus and are ingested.

At 5 hours after shell loss, the cells of the digestive gland contain large lipoid vesicles, large vacuoles containing flocculent material, and phagocytic vacuoles containing whole larval and algal cells (Fig. 30b). By 48 hours, neither the stomach nor the digestive gland contain material within their lumina (Fig. 30c), and the lipoid vesicles have disappeared. Occasional phagocytic vacuoles are still recognizable (Figs. 26b, 30c). At this stage, the nuclei of the digestive gland cells are very large and contain prominent nucleoli; numerous, darkly stained vesicles are apparent in some of the cells (Fig. 30c). These are histologically similar to zymogen cells within the digestive gland of older juveniles Fig. 30d). In 3 day old juveniles, material is present in the lumen and in phagocytic vacuoles of the digestive gland (Figs. 33d, 34d). Microscopic examination of 4 day old juveniles indicates that the digestive gland has become elaborated into five lobes; the arrangement which is typical of dorid nudibranchs.

The epithelium of the intestine is not visibly altered at metamorphosis.

b. Muscle Systems

During the first one-half hour after shell loss, the larval retractor muscle begins to disintegrate. In histological sections, this process is first apparent as a disorganization in the orientation of the muscle fibers (Fig. 31). By 4 hours after shell loss, the retractor muscle cannot be identified in one micron sections.

Although the retractor muscle is destroyed at metamorphosis, the sub-epidermal muscle fibers in the foot are retained and further elaborated. After formation of the notum, an extensive system of sub-epidermal fibers differentiate within this structure and these merge with those of the foot (Fig. 32c).

The alimentary tract also becomes muscularized during metamorphosis. In 4 day old juveniles, muscle processes are evident around both the stomach (Fig. 32a), and the digestive gland (Fig. 26c), and circular muscles eventually envelop the esophagus and intestine. The latter are especially well developed around the rectum (Fig. 32b). The development of the buccal musculature has been previously mentioned.

c. Nervous System

The ganglia of the nervous system do not undergo fusion at metamorphosis. The identity of each of the five pairs is retained and all but the optic ganglia undergo considerable enlargement following metamorphosis. Since this growth of the ganglia is not accompanied by a lengthening of the connectives, the latter eventually form only short, narrow links between the spherical, ganglionic masses. Following metamorphosis, the positions of some of the ganglia are shifted as the buccal mass differentiates and grows. As the dorsal swelling of the buccal pump increases in size, the cerebral commissure, and its attached cerebral ganglia, are displaced posteriorly. The pedal ganglia, which are connected to the cerebrals by cerebo-pedal connectives, also move posteriorly so that the pedal connective comes to lie between the radular sac and the esophagus. In the veliger, this connective lies anterior to the radular sac rudiment. Since the connectives do not lengthen as the esophagus-buccal mass region grows, the pedal ganglia come to lie lateral, rather than ventral, to the esophagus. Each pedal ganglion gives rise to a large pedal nerve which extends beneath the digestive gland. The pleural ganglia also shift posteriorly. They become situated posterior to the cerebrals, against the dorso-lateral walls of the esophagus. As mentioned previously, the pleural ganglia do not fuse with the cerebrals following metamorphosis. Since the position of the buccal ganglia remains unaltered, this pair becomes anterior to the main bulk of the cerebro-pedal complex.

A sixth pair of ganglia, the rhinophorals, are evident within 1 week after shell loss. Subsequently, the epithelium of the notum begins to bulge above each of these ganglia. These protuberances eventually differentiate into the rhinophores and the surrounding rhinophoral sheaths.

d. Kidney, Branchiae, and Heart

Following shell loss, the remaining larval kidney cells and the adult kidney rudiment are located in the posterior region of the visceral mass (Figs. 33a, 33b), to the right of the intestine (Fig. 33c). The type b, c, and d larval kidney cells are eventually lost, apparently by resorption. The vacuolate cells of the adult kidney rudiment encompass a small internal cavity at 5 hours post-shell loss (Fig. 33b). The size of the intracellular vacuoles, and particularly the cavity progressively enlarge during the following days (Fig. 33d). Eventually, the kidney extends anteriorly to the level of the cerebral ganglia, and occupies most of the dorsal region of the nudibranch in the region posterior to the stomach (Fig. 33e). The kidney duct opens into the right, lateral wall of the rectum.

The first pair of branchiae (Fig. 32b), and the pulsatile activity of the adult heart become apparent late in the second week after metamorphosis.

e. Gonad

As the left digestive gland is rotated anteriorly at metamorphosis, the ventral extremity of this gland, and the associated cells of the gonadal rudiment, become directed posteriorly. Therefore, the primordial germ cells are located at a terminal position in post-larvae fixed immediately after shell loss (Fig. 34a). As the reflected periphery of the mantle fold fuses with the pedal epithelium, the gonadal cells progressively migrate anteriorly, between the notum and the digestive gland (Figs. 34b, 34c). In 3 day old juveniles, the rudiment is located dorsal to the digestive gland, immediately posterior to the stomach (Fig. 34d). At this time, the germ cells have become altered in histological appearance. Although the large nuclei and prominent nucleoli persist, the cytoplasm stains homogeneously and densely (Fig. 34d). The cells of the prospective gonad multiply continuously throughout juvenile development, eventually forming a large mass of cells on the dorsal surface of the digestive gland (Fig. 34e). The anterior genital complex differentiates on the right, antero-lateral portion of the juvenile, separate from the gonadal rudiment.

f. Glands of the Foot and Mantle

The glandular elements of the mantle (prospective notum) and of the foot, undergo considerable metamorphic change. For at least 1 day after shell loss, all the pedal glands of the stage IV larvae are present (Figs. 26a, 34c), although the ducts of the propodial, metapodial, and accessory metapodial glands are filled with their secretory products (Figs. 34a, 34c, 26a). At 48 hours after shell loss, the metapodial glands are no longer present (Fig. 23d). At this time, the pedal groove glands and the type II unicellular mucous glands have also disappeared. The type I unicellular mucous glands are retained, and occupy dorso-lateral positions on the foot. The propodial glands are also retained in post-metamorphic stages, but their size relative to the total size of the foot, is reduced. The metapodial, and accessory metapodial glands are replaced by numerous, smaller multicellular glands. The ducts of these glands open over the entire sole of the foot and the secretory product is histologically similar to that of the propodial glands (Fig. 26c).

The unicellular mucous glands, which differentiate in the columnar epithelium of the larval mantle, are retained for 3 to 5 days after shell loss. At 10 days of age, these cells are no longer present. Instead, the large cuticle-secreting glands of the notum have become apparent, and a thin cuticle is present over the surface of the notum (Fig. 35a). Older juveniles and adults were occassionally observed with the cuticle partially disconnected from the surface of the notum.

E. GROWTH AND FEEDING OF JUVENILES

During the first four weeks of benthic life, the growth rate curve for D. steinbergae is sigmoidal (Fig. 36). Growth during the initial week after shell loss is slight. Figures 37a, 37b, and 37c, depict animals at three successive ages during this initial growth period. During the second week, the juveniles undergo a period of accelerated growth. Figure 37d shows an animal at 8 days of age, while the animal in Figure 37e is 13 days old. The pigment, which is present in the notum of the latter animal, typically becomes apparent at 12 days after shell loss. A high rate of linear growth occurs during the following 10 days. The growth rate subsequently decreases beginning at the onset of sexual maturity at 22 to 26 days of age. Prior to sexual maturity, much of the energy obtained by the feeding juvenile is presumably directed into growth, while gamete production demands a greater percentage of this energy after sexual maturity is attained.

The growth of laboratory reared animals was followed up to 40 days of age, at which time the average notum length was 6.0 mm. However, 7 mm. animals were frequently found in the field and 8 and 9 mm. animals, although rare, were also collected. Figure 37f shows two size classes of adult D. steinbergae.

The benthic phase of the life cycle, which includes the crawling veliger, the post-larva (after shell loss but before feeding), the juvenile, and the adult, differ not only in morphological criteria, but also in behavioral characteristics. Of these four stages, the post-larvae and the young juveniles are the most sedentary. In the presence of a plentiful supply of M. villosa, the first 3 weeks of post-metamorphic life are spent almost exclusively on the bryozoan, and feeding activity is continuous. The low rate of activity in post-larvae and juveniles contrasts with that of the crawling veliger stage. The latter individuals crawl rapidly over the surface of the bryozoan and the algae until just before metamorphosis.

The sluggish nature of young juveniles is not due to a lack of crawling proficiency, as juveniles isolated from M. villosa will become active. In these instances, crawling is occasionally interrupted by periods of rearing behavior, during which the animal raises the anterior portions of the body off the substratum and pivots on the posterior end of the foot. The rhinophores are fully extended during this period. When these juveniles are replaced on a colony of M. villosa, active crawling ceases, and the animals commence feeding.

The importance of continuous feeding to juveniles was illustrated by an experiment in which four, 12 day old juveniles were isolated from M. villosa for a period of 6 days. The results, shown in Figure 38, indicate that growth is arrested within 1 day after isolation. After reintroduction of M. villosa, three of these animals resumed growing within 4 days and one died.

As juveniles approach adulthood, they exhibit a progressively greater tendency to wander from the surface of the bryozoan. Adults were frequently observed crawling on the adjacent algal surface and on the glass bowl. Therefore, the percentage of time spent feeding is greatest in young juveniles and least in adults.

In addition, the attainment of sexual maturity coincided with the onset of congregating behavior. Both in the laboratory and in the field, adults of various sizes were often grouped in aggregates of 3 to 4 individuals. Frequently, some of these individuals were copulating.

Juveniles begin feeding on zooids of M. villosa between the second and third day after metamorphosis. Although the average zooecium length of M. villosa zooids is approximately 0.6 to 1.0 mm., the notum length of juveniles is only 0.20 to 0.22 mm. at the time of their first zooid meal. While feeding on their first zooid, young juveniles situate themselves on the frontal membrane of the zooid or on a zooecium wall with the anterior end extending onto the frontal membrane. Rhythmic movements of the radula can be seen. The zooid appears undisturbed at the onset of the attack, it continues to extend through the aperture and exhibits normal feeding movements of its lophophoral tentacles. Eventually, however, the zooid begins to repetitively retract into its zooecium and within 2 hours after the initiation of the attack it remains contracted. Within the subsequent 5 to 10 hours, the outlines of the lophophoral tentacles become indistinguishable. Instead, the zooecium appears to be filled with a mass of structureless, flocculent material (Fig. 39). By allowing young juveniles to feed on colonies of _M_. _villosa_ which encrusted glass slides, it was possible to observe the feeding process with a compound microscope and transmitted light. Using this technique, numerous spherical cells could be seen undergoing rapid, erratic movements within the zooecium of zooids being attacked. Since the integrity of the lophophore is lost during the feeding activities of young juveniles, these spheres were presumed to be the ciliated cells of the lophophoral tentacles which had become dissociated. During the ensuing 20 to 30 hours, the disrupted tissue of the zooid gradually disappears from the zooecium. Rhythmic movements of the radula can be seen throughout this period. Of those juveniles observed, a period of 36 to 43 hours was required to consume each of their first two zooids (Table 4). One of the juveniles abandoned its second zooid meal when it was only half consumed. Of the seven other, three consumed their prey completely, while four left small amounts of tissue within the zooecium. However, in none of these cases did juveniles ingest the retractor muscles of the zooid. These remained attached to the inner wall of the empty zooecium.

During the feeding process, juveniles were never observed inside the frontal membrane of the zooid and the oral area of the nudibranch remained at the same position on the frontal membrane throughout the feeding process. Presumably, the contents of the zooecium are removed through a hole rasped in the frontal membrane by the radula.

At 9 days of age, the consumption period per zooid is reduced to 11 to 15 hours (Table 4). The major reason for this increase in feeding effeciency appeared to be a reduction in the time required to ingest the dissociated tissues of the zooid.

A large increase in consumption rate occurred between 9 day old and 13 day old juveniles. Juveniles in the latter age class consume zooids at an average rate of one per 1.7 hours. In addition, the feeding activities of 13 day old juveniles differ from those of younger stages, in that extensive dissociation of the zooid tissues does not occur prior to ingestion. The lophophore and digestive tract of the zooids is removed from the zooecium in chunks. Unless mechanically disturbed, animals older than 13 days were never observed to abandon a zooid before completely ingesting its contents.

The feeding effeciency of D. steinbergae continues to increase as the animal grows. Animals measuring 3.5 mm. in length (approximately 21 days old) can consume zooids at a rate of 4 to 9 minutes per zooid.

More detailed observations of the feeding process were made on older juveniles and adults, in which the buccal mass was large enough to observe. The feeding process in these animals conformed to McBeth's (1968) description. After probing the zooids with the oral tentacles, the oral lips are pressed over the surface of a frontal membrane. Subsequently, rasping movements of the radula and repetitive dilations of the buccal pump begin simultaneously. Shortly after, the tissue of the zooid can be seen passing down the esophagus to the stomach.

The number of zooids consumed over a 24 hour period was determined for five size classes of D. steinbergae. Each animal which was examined was transferred to a piece of algae bearing recently collected, intact colonies of M. villosa. The locations of all degenerate zooids were recorded prior to the start of the observation period. The animals were regularly observed throughout the experimental period, and at the end of 24 hours, the number of empty zooecia was recorded.

The results, illustrated in Figure 40, indicate that the greatest increase in daily consumption of zooids occurs between the 2.0 mm. and 3.0 mm. size classes. Although only one size class greater than 3.0 mm. was tested, the results from this group indicate that consumption continues to increase in animals greater than 3.0 mm., but the rate of this increase is lower, relative to the previous period. Finally, age dependent consumption during any 24 hour period is relatively uniform within each of the younger size classes (0.28 mm., 0.50 mm., 1.07 mm.), but becomes highly variable in animals measuring 3.0 mm. (20 days old) and greater.

DISCUSSION

A. LARVAL DEVELOPMENT

Newly hatched, planktotrophic nudibranch larvae are neither morphologically nor neurologically capable of undergoing the transformation to the benthic stage (Hadfield, 1978). Growth and differentiation must take place to prepare the veligers for the metamorphic process and for the post-metamorphic life style. The requisite energy for these morphogenetic events must be derived from the plankton. Therefore, the larvae are presented with the conflicting tasks of progressively differentiating prospective adult structures, which are designed for a benthic life style, while maintaining their efficiency as feeding, pelagic dissemules (Fretter, 1969; Switzer-Dunlap, 1978).

From the studies of Kriegstein (1977; 1977b), Switzer-Dunlap and Hadfield (1977), and Switzer-Dunlap (1978) on aplysiid veligers, and Chia and Koss (1978) and Perron and Turner (1977) on nudibranch veligers, we have some appreciation for the sequential development of planktotrophic opisthobranch larvae. However, histological study of organogenesis was not attempted in the latter four studies, and Kriegstein (1977; 1977b) studied only the histogenesis of the nervous system. Several histological studies of lecithotrophic opisthobranch veligers have been reported (Thompson, 1958; 1962; Smith, 1967). However, since these are non-feeding larvae, they are not subject to the dual demands of both feeding and differentiation. It is reasonable to suppose that the midgut of lecithotrophs may differ considerably from that of planktotrophs. Indirect evidence for this is derived from Thompson's (1958; 1962) papers on the development of the lecithotrophic nudibranch larvae of _Adalaria proxima_ and _Tritonia hombergi_. Although the digestive gland of the former larva was apparently capable of phagocytizing algal cells, the gut is described as a uniformly ciliated tract composed of basically undifferentiated cells. Since Thompson (1958) describes the tissues of _Adalaria proxima_ in considerable detail, it seems unlikely that he might have overlooked the specialized ciliated regions, the cuticle, and the refractile rods, had they been present. In a histological study of the direct development of _Retusa obtusa_ (Cephalaspidea), Smith's (1967) description of the gut is similar to that of Thompson's (1958) for _Adalaria proxima_.

Although Thompson (1959) has studied the gut structure of several species of planktotrophic nudibranch larvae, he used only young veligers. Therefore, possible developmental changes in the midgut were not examined.

Thompson (1959) did not allude to any differences between the alimentary tract of Adalaria proxima, and that of the planktotrophic veligers which he subsequently studied.

The results of this study indicate that all the structural components of the gut of newly hatched D. steinbergae larvae are retained throughout the larval period. The structure of this gut, and the treatment of food by the veligers, is in greater conformance with the descriptions of Fretter and Montgomery (1968) for prosobranch veligers, than with those of Thompson (1959) for planktotrophic nudibranch veligers. Although Thompson (1959) described the specialized band of dense ciliation in the dorsal stomach, he states that a cuticle is absent. In addition, Thompson's (1959) Figure 6 shows the food bolus extending throughout the length of the stomach. In prosobranch larvae (Fretter and Montgomery, 1968), in the larvae of Rostanga pulchra (Koss, 1978; personal communication), and in D. steinbergae, a cuticle is present in the ventral portion of the stomach and the food bolus rotates only in this region in living veligers.

Without knowledge of the physical or chemical properties of the cuticle or the refractile rods, their functional role is only speculative. Since food particles are continuously rotated against this portion of the ventral stomach, the cuticle and refractile rods may aid in mechanical disruption of food particles (Fretter and Montgomery, 1968) or they may protect the gastric wall from abrasion. In either case, the cuticle would be analagous to the gastric shield of ~~primitive~~ prosobranchs (Morton, 1958; Fretter and Montgomery, 1968). Thompson (1959), without apparent justification, states that the refractile (hyaline) rods are likely the site of some enzymatic activity and therefore serve a function similar to that of a style. A conventional crystalline style is present in the gut of larval oysters (Yonge, 1929; Millar, 1955), but such a structure has not been reported in gastropod larvae. However, Fretter and Montgomery (1968) report that fecal particles of prosobranch veligers are agglutinated by secretory products and wound into spiral coils by the ciliary currents of the style sac (dorsal stomach). Similarily, in D. steinbergae, a transient rod of apparent mucous material is present in the dorsal stomach and is rotated by the dense ciliation in this region. This structure has also been seen in the planktotrophic veliger of Tritonia diomedea (Kempf, 1976; personal communication). In live veligers, and in histological sections, particles of apparent fecal material are embedded in this mucous rod. Therefore, it seems likely that the rod is analogous to the protostyle of some archeogastropods (Morton, 1951). In these primitive gastropods, the rotation of the protostyle by the cilia of the style sac helps circulate the contents of the ventral stomach, and fecal material is progressively wound around its mucous matrix. The cilia of the intestinal groove and of the intestine gradually transport this complex of wastes and mucus into the intestine. Ideally, primitive prosobranchs and planktonic gastropod veligers both feed continuously on particulate, herbivorous material. Therefore, it is perhaps not surprising that a great deal of similarity is found in the design of the stomach and the mechanism of food treatment.

The digestive gland of oyster larvae (Millar, 1955) and prosobranch larvae (Fretter and Montgomery, 1968) is muscularized. The muscles produce pulsations of the digestive gland which draw food particles into the lumen of the gland. However, Thompson (1959) states that movement of particles between the stomach and the digestive gland of nudibranch veligers is accomplished only by ciliary means. Observations on D. steinbergae support Thompson's (1959) statement. Contractions of the left digestive gland were not observed in live veligers and muscle fibers were not found in association with this organ in sectioned material.

Fretter and Montgomery (1968) report that whole algal cells were never observed within the digestive gland cells of monotocardian prosobranch larvae. Millar (1955) made the same observation on the larvae of Ostrea edulis and contends that digestion is exclusively extracellular. However, Thompson (1959) found whole algal cells within the digestive gland cells of Archidoris pseudargus. Histological examination of Rostanga pulchra veligers reveals the same phenomenon; as many as 4 to 5 algal cells can be discerned within a single phagocytic vacuole (personal observation). However, intracellular digestion is not apparent in D. steinbergae larvae, although numerous whole algal cells are evident in the lumen of the left digestive gland. Instead, the cells of the digestive gland contain large vacuoles filled with a flocculent material which is histologically similar to the background substance within the lumen of the gland.

Several observations suggest that the dense purple-blue vesicles (Richardson stain) in the digestive gland of D. steinbergae may embody lytic enzymes which act extracellularly on ingested algal particles. Firstly, these vesicles are present in the gland cells from hatching until the onset of metamorphosis. They must, therefore, have a functional role throughout larval development. Secondly, the vesicles were sometimes seen in the lumen of the gland (Fig. 9b), suggesting that they may function extracellularly. These observations do not conclusively prove that the vesicles are enzymatic. However, these vesicles are not present in the digestive gland cells of

Rostanga veligers, which phagocytize whole algal cells. If the dense vesicles are indeed enzymatic, the ingested algal cells are likely subjected to a combination of both mechanical and chemical disruption, and the resultant products phagocytized by the digestive gland cells.

During the period of larval development in which the large, yolky cells are present in the digestive gland, the number of differentiated, digestive cells increases. Subsequent growth of the gland, following the disappearance of the undifferentiated cells, appears to result from a progressive enlargement of the constituent cells. It seems possible that the undifferentiated cells are digestive gland stem cells. Similar cells were also noted by Thompson (1959) in the digestive glands of a number of species of young planktotrophic nudibranch veligers.

As the planktotrophic larvae of D. steinbergae age, the digestive gland appears to assume an additional function other than digestion and absorption of algal food. If the green-staining vesicles are indeed lipoid, the gland likely functions as a nutrient storage organ during the latter stage of larval development. Similar vesicles (at least in external appearance) have been seen in the older planktotrophic larvae of the nudibranchs Rostanga pulchra (Koss, 1978; personal communication), Tritonia diomedia, and Melibe leonina (Kempf, 1976;

personal communication), and of aplysiids (Switzer-Dunlap, 1978). Switzer-Dunlap (1978) also presumes these vesicles have a lipid content and she suggests that they provide nutriment during the non-feeding metamorphic phase.

During larval development, the only components of the adult digestive tract which begin to differentiate are those which will not interfere with the larval feeding process. The radular sac, radular teeth, and precursors of the odontophore musculature make their appearance because these develop apart from the main tube of the esophagus, within an isolated outpocketing of the ventral esophageal wall.

Since the oral lip glands, which become functional during the feeding activities of the benthic stage, can differentiate completely independently of the larval alimentary tract, it is perhaps not surprising that these appear prior to metamorphosis. Nevertheless, these glands have not been reported in competent veligers of aplysiids (Kriegstein, 1977; 1977b), Adalaria proxima (Thompson, 1958), or Tritonia hombergi (Thompson, 1962).

No changes which might anticipate the benthic life style are apparent in the larval stomach. During larval life, the stomach must deal with particulate food. As in the gut of archeogastropods, mucus and cilia act in concert to process this type of food (Morton, 1951). In contrast, the adult gut is a simple muscular sac (MacFarland and O'Donoghue, 1929) designed to deal with semi-fluid bryozoan tissue. Apparently, these two temporally separated roles of the stomach require incompatible structural correlates. Thus, the formation of the benthic stage midgut does not begin until metamorphosis.

In the long-term planktotrophic larvae of Aplysia californica, both the cerebral and pedal ganglia are present in the hatching veliger (Kriegstein, 1977). In D. steinbergae, only the former pair are present. Presumably, the cerebral ganglia are capable of directing all the essential larval behaviors (excluding those which will enable it to settle and metamorphose), since newly hatched veligers of D. steinbergae are capable of swimming, and acquiring and processing food particles. Carter (1926, 1928) and subsequently Mackie et al. (1976) have shown that the activity of the velar cilia of nudibranch and prosobranch veligers, respectively, is controlled by nerves arising from the cerebral ganglia. In addition, the cerebrals innervate the statocysts (Smith, 1967; Kriegstein, 1977).

In D. steinbergae, the differentiation of the remainder of the ganglia is correlated with the functional development of the structures which they innervate[1]. The optic ganglia differentiate as the eyes form and the pleural ganglia are formed just prior to the onset of mantle retraction, a process which involves coordinated action of the mantle retractor muscles, and subsequently enables functioning of the larval retractor muscle. Although the pedal ganglia are recognizable at stage II, they do not become differentiated into medullae and cortexes until the subepidermal pedal muscles and the multicellular pedal glands begin to develop at stage III. Similarly, the buccal ganglia are not apparent until the radular sac evaginates and the precursors of the odontophore musculature begin to organize. The buccal ganglia of _D. steinbergae_ do not differentiate in asymmetric positions, as noted by Thompson (1958) for the dorid, _Adalaria proxima_. In the planktotrophic larvae of _Aplysia californica_ (Kriegstein, 1977), the lecithotrophic veligers of _Adalaria proxima_ (Thompson, 1958) and _Tritonia hombergi_ (Thompson, 1962), and the directly developing cephalaspidean, _Retusa obtusa_ (Smith, 1967), the development of the nervous system is also a gradual process, with the individual ganglionic pairs differentiating according to a specific temporal pattern. In these species, a basic correlation is apparent between the appearance of each ganglionic pair and the functional differentiation of the innervated structure.

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[1] Although details of innervation were not studied in _D. steinbergae_, it is assumed that the pattern is similar to those opisthobranchs in which this has been elucidated (Bullock and Horridge, 1965).

Switzer-Dunlap (1978) has stated that "the segments of larval life of aplysiids concerned with growth on one hand, and development to metamorphosis on the other, are temporally separated". Her observation is supported by the work of Chia and Koss (1978) on the dorid nudibranch Rostanga pulchra. This is equally true of D. steinbergae, as evidenced by the fact that all of the adult rudiments differentiate after cessation of shell growth. However, in the previously studied opisthobranch species, the mantle does not retract until two-thirds of the way through the obligatory larval phase, while in D. steinbergae the mantle is retracted before the midpoint of the larval phase (Fig. 4). As a possible consequence, the maximal shell size attained by D. steinbergae veligers is approximately half that of the aplysiids and Rostanga pulchra. The precocious retraction of the mantle fold in D. steinbergae is paralleled by a correspondingly early development of the pleural ganglia. In Aplysia californica, the pleurals develop just prior to mantle retraction but considerably later than the appearance of the optic ganglia (Kriegstein, 1977). In D. steinbergae, the optic ganglia differentiate at a stage which is temporally similar to the stage of eye differentiation in *A. californica* (12 days in *D. steinbergae*; 14 days in *A. californica*). However, in *D. steinbergae*, the differentiation of the pleural ganglia and the process of mantle retraction occur simultaneously with the development of the eyes. It would appear that aplysiids and the dorid nudibranch, *Rostanga pulchra*, place a greater percentage of the total energy acquired by the larvae into shell and body growth, while in the veliger of *D. steinbergae*, the majority of the acquired energy is apparently directed into differentiation of adult rudiments. There is considerable overlap between *R. pulchra*, the aplysiids, and *D. steinbergae* in the type of benthic stage organ rudiments which are developed during the larval stage. However, perhaps the most signifigant difference between *D. steinbergae* veligers and the other opisthobranchs, in this respect, is the development of the gonadal rudiment in *D. steinbergae*. The only previous report of the occurrence of a gonadal rudiment in the larval stage of an opisthobranch is that of Thiriot-Quievreux (1970) for the planktotrophic veliger of the pteropod, *Cymbulia peroni*. In *D. steinbergae*, this rudiment is recognizable at the time of mantle retraction and attains a considerable size by stage IV. The differentiation and growth of the gonadal rudiment may require a substantial amount of energy which is perhaps obtained at the expense of shell growth.

In a comparative study of four species of aplysiid veligers, Switzer-Dunlap and Hadfield (1977) noted that the maximal size of the larval shells was a species specific characteristic. These interspecific differences in shell size were not correlated with the shell size at hatching, with the adult size, or with the length of larval life (Switzer-Dunlap, 1978). In attempting to explain the differences, Switzer-Dunlap (1978) proposed that the size attained by the larval shell, and thus the maximal size which the larval body can attain, might be related to the ability of the juveniles to exploit their respective post-metamorphic food sources.

Young juveniles of D. steinbergae possess a mechanism which enables them to feed on M. villosa zooids, despite the considerable size differential between the predator and prey. If the selective pressure for optimal larval size in D. steinbergae is indeed a function of its ability to exploit M. villosa zooids, then the modified feeding strategy of juveniles of this species may have rendered the production of a larger shell, and consequent body size, superfluous. Since the free-swimming larval stage is probably a much more hazardous phase in the life cycle than the benthic stage, one might expect that the additional time required to produce a shell and body size which is larger than benthic survival demands, would be selected against.

Previous authors have referred to the mantle retractor muscles as the mantle muscles (Hurst, 1967; Williams, 1971) and the retractor muscles (Bridges and Blake, 1972). Although not mentioned in the text, muscles which originate on the perivisceral membrane and insert on the mantle fold are shown in published figures of *Aplysia californica* (Kriegstein, 1977b), *Rostanga pulchra* (Chia and Koss, 1978), *Phyllaplysia taylori* (Bridges, 1975), and *Adalaria proxima* (Thompson, 1958). The signifigance of these muscles has not been previously stated. In *D. steinbergae*, the mantle retractor muscles appear to function in withdrawing the mantle fold from the aperture of the larval shell at the end of shell deposition.

Among nudibranchs, the further development of the retracted mantle fold may follow one of two routes. The mantle cells either remain squamous, as in *Phestilla sibogae* (Bonar and Hadfield, 1974) and *Aeolidella alderi* (Tardy, 1970) or the cells hypertrophy, as in *Adalaria proxima* (Thompson, 1958), *Tritonia hombergi* (Thompson, 1962), and *Rostanga pulchra* (Chia and Koss, 1978). The retracted mantle of *D. steinbergae* belongs to the second category. However, as the mantle fold cells hypertrophy in *A. proxima*, *T. hombergi*, and *R. pulchra*, the entire fold becomes reflected upon itself within the shell cavity. In *D. steinbergae*, proliferation and growth of the mantle fold cells is not accompanied by reflection. Instead, the hypertrophied mantle fold remains in its post-retraction position, encompassing the mantle cavity on the dorsal and right dorso-lateral sides of the veliger.

The upward swimming response, which is exhibited immediately after hatching, has been reported in both lecithotrophic species (Thompson, 1958; 1962) and in planktotrophic veligers (Chia and Koss, 1978; Hurst, 1967; Hadfield, 1963). In some cases, a positive phototaxis may contribute to this behavior (Harris, 1975; Hurst, 1967; Chia and Koss, 1978). However, since this behavior will occur in the absence of light in D. steinbergae, it is more likely a negative geotaxis, as suggested by Thompson (1958) for Adalaria proxima and Switzer-Dunlap and Hadfield (1977) for aplysiids. Alternatively, the behavior may be the result of a programmed hyperactivity of the velar cilia. In short-lived, lecithotrophic species, the upward swimming likely facilitates dispersal. In planktotrophic veligers, the behavior may function to bring the larvae into the plankton-rich, surface layer of the ocean.

Perron and Turner (1977) reported that competent veligers of Doridella obscura were never observed to crawl in the absence of the adult food, Electra crustulenta. This contrasts with the behavior of the competent, lecithotrophic veligers of Phestilla sibogae (Bonar and Hadfield, 1974), Adalaria proxima (Thompson, 1958), and Tritonia hombergi (Thompson, 1962), which frequently crawl between periods of swimming. Although crawling on the glass culture bowls was uncommon in D. steinbergae, it seemed to be stimulated by agitation of the culture water. When the competent veligers are swept past a solid surface, they might detect the relative movement between themselves and the solid surface, possibly as a shear force aginst the long tufts of cilia which protrude from the apical and lateral edges of the foot. Bonar (1978) gives evidence which suggests that these tufts are mechanoreceptors. Reception of this type of stimuli may induce crawling behavior in competent D. steinbergae veligers.

As water currents flow past any solid substrate, such as the fronds of large kelps, particles suspended within the water will be jostled against the surface. Immediate crawling upon such contacts would be advantageous to D. steinbergae veligers as any solid surface might have encrustations of M. villosa. Newly metamorphosed juveniles were found on field collected fronds of kelp which bore widely scattered colonies of M. villosa consisting of not more than 50 zooids per colony. Due to the small size of these colonies and the continuous movement of water around the algal fronds, it seems unlikely that the crawling behavior which preceded metamorphosis in these instances, was dependent upon the reception of a stimulatory chemical cue.

Nevertheless, crawling behavior by D. steinbergae veligers seems to be definitely enhanced by the presence of M. villosa. It is not known whether this effect is mediated by reception of a dissolved chemical, or whether the larvae actually contacted the colony before crawling was initiated.

B. INDUCTION OF METAMORPHOSIS

Crisp (1974) has proposed a number of criteria which must be fulfilled to prove chemical interaction between an animal (species A) and its substrate (species B). These have been restated by Hadfield (1977) as follows: 1. In the field, the benthic stage of species A must always be found in association with species B. 2. In laboratory experiments, the competent larvae of species A must exercise a preference for settlement on or near species B, when a number of choices are available. 3. A chemical component can be isolated from species B which can confer inductive potency on an otherwise unsuitable settlement substrate for species A.

The field observations and the preliminary laboratory experiments, which have been reported here, indicate that Doridella steinbergae conforms to all three criteria.

In the first instance, the highly localized field distribution of D. steinbergae is correlated with the presence of Membranipora villosa. The dorids were never seen on fronds of Nereocystis luetkeana, Laminaria saccharina, or Costaria costata which were not epiphitized with M. villosa. In addition, Seed (1976) found that the population densities of D. steinbergae on fronds of L. saccharina were distributed according to the percent coverage by Membranipora sp. along the length of the frond.

Secondly, the results of the laboratory experiments presented here indicate that competent veligers of D. steinbergae will metamorphose only in the presence of M. villosa, regardless of the substrate colonized by this bryozoan. Competent larvae did not metamorphose on the algal thallus alone, on glass slides covered with a microbial and diatom film, or on clean glass surfaces[1].

During a study of the western Atlantic species, Doridella obscura, Perron and Turner (1977) found that metamorphosis was induced only by Electra [Membranipora] crustulenta. These investigators tested the inductive capacity of three other bryozoan species, including Membranipora tenuis, and found that none were effective.

Although the experiment designed to test the third criterion was very preliminary, the results do suggest that a chemical, rather than a physical characteristic of M. villosa provides the metamorphic inducer. However, it is not possible to state whether this chemical can be sensed in a dissolved form, or whether it must be in the form of an adsorbed layer. Hadfield (1977) has shown that the metamorphic inducer of the aeolid nudibranch, Phestilla sibogae, can be perceived in a dissolved state.

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[1] although one larva did metamorphose while in culture, the percentage which this represents is insignifigant relative to the number which responded to M. villosa. Harris (1975) has also reported that 1 out of 300 veligers of Phestilla sibogae was observed to metamorphose spontaneously.

With respect to the process of metamorphic induction, two notable differences exist between Phestilla sibogae and Doridella steinbergae. Firstly, when competent veligers of P. sibogae are placed in bowls containing Porites compressa, metamorphosis will occur either on the coral tissue or on the glass bowl, apparently indiscriminately (Harris, 1975). However, competent veligers of D. steinbergae always metamorphose in direct contact with M. villosa if the latter is present. The only exceptions to this were the one incidence of spontaneous metamorphosis and the two larvae which metamorphosed in sea water conditioned with M. villosa.

The second difference between the two nudibranchs is in the duration of the latency period. This is defined as the time period which extends between the introduction of the larvae to the inductive stimulus, until the first visible signs of metamorphosis (Hadfield, 1978). When veligers of P. sibogae are exposed to extracts of P. compressa, the latency period is usually not less than 12 hours, although less than 24 hours (Bonar and Hadfield, 1974; Hadfield, 1978). Although the latency period in D. steinbergae is variable, the majority of the larvae metamorphosed within 5 hours after exposure to intact colonies of M. villosa. As a result of studies on

P. sibogae, Hadfield (1978) has stated that "the rapid and apparently triggering effect of molluscan inducers suggests a major role of the nervous system, both in receiving the stimulus and transmitting the effect through the larval body". The very short latency period exhibited by D. steinbergae supports this theory even more dramatically than does P. sibogae.

Although all larvae within each culture bowl were raised under the same conditions, their response to M. villosa was not uniform. Considerable variation in the latency period was observed and some larvae never metamorphosed. Hadfield (1977) also noted this phenomenon; extracts of P. compressa never effected 100 percent metamorphosis in a batch of P. sibogae larvae. Hadfield (1977) found that individual larvae have separate thresholds for minimal inducer concentration and minimum exposure time to the inducer. It is also possible that some of the veligers, particularly in species which must be cultured through a long planktotrophic period, develop morphological or neurological abnormalities which block their ability to respond to the inducer. Non-uniform health between the veligers within each culture was evidenced by the fact that 20 to 30 percent of the veligers did not even survive to competency (culture technique II).

The capacity for delayed metamorphosis in planktotrophic opisthobranch veligers has been studied by Kriegstein _et al_. (1974) for _Aplysia californica_, and Chia and Koss (1978) for _Rostanga pulchra_. When veligers of _A_. _californica_ were maintained in culture for 4 weeks after the attainment of competency, they exhibited 85 percent metamorphosis when exposed to the alga _Laurencia pacifica_. During the 4 week delay period, the level of differentiation and the behavior of the veligers did not change (Kriegstein _et al_., 1974). At 3 weeks after the attainment of competence, veligers of _R_. _pulchra_ displayed greater than 80 percent metamorphosis when exposed to the sponge, _Ophlitaspongia_ (Chia and Koss, 1978). However, the lecithotrophic veligers of _Adalaria proxima_ began to exhibit abnormalities at one week after the attainment of competency (Thompson, 1958). As a result, these larvae began to lose their ability to respond to the bryozoan, _Electra pilosa_.

Although veligers of _D_. _steinbergae_ displayed optimum response to _M_. _villosa_ even after a 9 day delay period, those which had been delayed for 15 days began to lose their ability to respond. However, this negative result does not merit a conclusive statement about the ability of oceanic larvae of _D_. _steinbergae_ to delay metamorphosis.

Among species of marine invertebrate larvae which excercise a substrate preference for settlement and metamorphosis, many progressively lower their threshold for the metamorphic response if the preferred substrate is withheld for a prolonged period. As stated by Crisp (1974) "the stimulus required for metamorphosis gradually diminishes with age, and the pre-settlement behavior pattern becomes shorter or is eliminated...". This phenomenon is not exhibited by D. steinbergae. Metamorphosis will be delayed indefinitely in the absence of M. villosa and the period of pre-settlement crawling activity is not affected by the delay. The absence of an age-dependent alteration in the latency period is shown in Figure 18. While a lowering of the metamorphic threshold might be adaptive in some species of marine invertebrate larvae, it would not be for nudibranchs. The buccal apparatus and digestive tract of nudibranchs are highly specialized for feeding on a particular type of prey (Forrest, 1953). Neurophysiological studies have revealed that opisthobranchs with specialized diets will exhibit a feeding response only after chemoreception of the prey organism (Kupfermann, 1974).

Although settlement and metamorphosis by nudibranch larvae is characteristically induced by the prey of the benthic stage (see Hadfield, 1978), there have been no previous reports of selection of a particular site on the inductive substrate. Harris (1975) has stated that veligers of Phestilla melanobranchia must settle on the calcareous skeleton of the coral, Dendrophyllia elegans, because direct settlement on the coral tissue resulted in veligers being eaten by the polyps. However, no evidence was given for active avoidance of the polyp tissue by the veliger. Competent veligers of D. steinbergae are too large to be potential food for M. villosa. On several occasions, veligers were seen to fall into extended lophophores, but the larvae were immediately ejected.

D. steinbergae veligers exhibit a marked preference for metamorphosing at the periphery of the bryozoan colony. The reason for this preference may have either a chemical or physical basis. Possibly, differentiating zooids produce the inducing chemical in higher concentrations than fully differentiated zooids. If so, one might speculate that only veligers with the lowest metamorphic thresholds would be induced by differentiated zooids. Alternatively, an organic surface film may mask the chemical nature of the fully differentiated zooids. On field collected colonies of M. villosa, a film of diatoms and debris is present on the older zooids which is thick enough to be seen macroscopically. However, since the undifferentiated zooids represent newly produced tissue, one might expect the film build-up to be least over this portion of the colony.

The selection of the colony periphery may also have purely physical justification. During the normal feeding activities of M. villosa zooids, the lophophore is periodically retracted into the zooecium. These retractions produce a considerable convulsion of the frontal membrane. Areas of such disturbance may be avoided by crawling veligers. Alternatively, the surface of brown algae or glass slides may provide a better adhesive surface for the secretions of the pedal mucous glands than does the bryozoan. Bonar and Hadfield (1974) have suggested that firm attachment between the foot and substrate is necessary to enable metamorphosing nudibranch larvae to withdraw the visceral mass from the shell. As will be discussed in the following section, histological examination of metamorphosing D. steinbergae larvae supports this theory. The importance of surface properties in facilitating various forms of bioadhesion has been reviewed by Baier et al. (1968).

The tendency for D. steinbergae larvae to settle within irregularities along the periphery of the bryozoan colony may be an extension of whatever encourages metamorphosis at the colony periphery. Alternatively, it may be mediated by an entirely distinct behavioral response. Some species of marine invertebrate larvae respond to a hierarchy of releasor stimuli immediately prior to metamorphosis. For example, larvae of the tube-dwelling polychaete, Spirorbis borealis, preferentially settle on the thallus of the brown alga, Fucus serratus (Williams, 1964), but they also express a secondary preference for settlement in grooves on the thallus (Wisely, 1960). Sensitivity to surface contour is termed rugotropism (Crisp and Barnes, 1954). Rugotropic behavior by D. steinbergae veligers, prior to metamorphosis, might be responsible for their affinity for the edge of the bryozoan colony and for indentations along this edge. Rugotropic settlement behavior is usually interpreted as an attempt to seek a protected location for vulnerable young juveniles (Crisp, 1974).

In addition to the above possibilities, several alternate hypotheses may be proposed for the adaptive value of veliger settlement at the periphery of the bryozoan colony. The blades of Laminaria saccharina and Costaria costata grow continuously throughout the summer from a point just distal to the stipe. Therefore, the basal portion of the blade supports only ancestrulae and young colonies of M. villosa. The number and size of the colonies progressively increases toward the older, more distal regions of the blade. At the apex of the blade, the colonies are confluent. As a result, the algal tissue in the distal regions cannot photosynthesize, eventually dies, and is progressively abraded away by wave action. This pattern of settlement and growth of Membranipora sp. on L. saccharina has been quantified by Seed (1976). Since confluent colonies do not have differentiating borders, veligers may avoid settling in these areas. The effect of this avoidance may discourage recruitment on areas of the frond which will soon be lost by attrition.

Since confluent colonies are also relatively old colonies, they are invariable coated with a considerable film of diatoms and debris. This material may impede the feeding activities of young juveniles by hampering their ability to penetrate the frontal membrane of the bryozoan zooids. By recruiting adjacent to differentiating zooids, the crawling larvae may insure a readily accessible food source for the first part of benthic life.

C. MORPHOLOGICAL CHANGES DURING METAMORPHOSIS

Bonar and Hadfield (1974), described the sequence of metamorphic events in Phestilla sibogae and stated that the order in which these events occurred was widely applicable. However, in a subsequent paper, Bonar (1978), noted that some species lose the velar lobes after, rather than before, shell loss. One of these exceptions is Doridella obscura. Perron and Turner (1977), imply that the velar lobes may be lost up to 2 hours after shell loss and the process is accomplished by resorption. In D. steinbergae, the velum may be lost either before or after shell loss, and the cells are lost by dissociation, rather than resorption. Most of the velar cells swim away from the metamorphosing larvae, while others may be ingested. In addition, the operculum and kidney structures do not dissociate in a fixed order, relative to the other events which occur at the beginning of metamorphosis.

Among opisthobranchs, the visceral organs of nudibranchs undergo the most dramatic reorganization at metamorphosis (Bonar, 1978). Visceral flexure is eliminated, detorsion takes place, and, in dorids, the stomach becomes positioned on the mid-dorsal surface of the digestive gland. In D. steinbergae, three major processes act in concert to effect the reorganization of the viscera.

These are: secretion of adhesive material by the multicellular pedal glands, contraction of the larval retractor muscle, and loss of the larval shell and operculum. The contraction of the larval retractor provides the motive force for the visceral rearrangements, while the loss of the shell, and the secretion of the pedal gland adhesive play facilitative roles.

Bonar and Hadfield (1974) and Bonar (1976) have suggested that, in order for the retractor muscle to pull the visceral mass out of the larval shell, this muscle must have a fixed support against which to contract. These authors propose that this support is provided by a firm attachment between the foot and the substrate, presumably effected by secretions of the pedal glands. The disappearance of the metapodial glands after shell loss supports the suggestion that the functional role of their secretions is involved in the metamorphic process. This was noted by Bonar and Hadfield (1974) for Phestilla sibogae and was also seen in D. steinbergae. Furthermore, the ducts of these glands are filled with secretory product during metamorphic stages.

The metapodial secretion does not solidify externally. During shell pivoting behavior and removal of the visceral mass from the shell, the veliger is able to swivel laterally on its foot (Fig. 21). Therefore, the adhesive secretion must be susceptible to shear forces yet it must possess considerable tensile strength.

Before the larval retractor can pull the visceral mass out of the larval shell, the trunk of the muscle must detach from the inner wall of the shell. Hadfield (1978) suggests that violent contraction of the larval retractor muscles in P. siboqae is responsible for rupturing the shell/muscle connection. However, the process may be aided by a separate mechanism. Bonar (1978) states that the muscle trunk is attached to the shell by specialized cells of the perivisceral membrane. Conceivably, these cells may be induced to break this connection at the onset of metamorphosis and a similar mechanism might exist to facilitate detachment of the operculum from the pedal epithelium. The removal of the restricting confines of the shell and operculum allows the visceral organs to be freely shifted, and allows dorso-ventral flattening of the post-larval body.

At the onset of metamorphosis in P. siboqae, the veliger pivots forward on its foot in such a way that only a restricted portion of the sole of the foot remains in contact with the substrate, and the outer apertural rim of the shell is forced against the substrate (Hadfield, 1978). Bonar and Hadfield (1974) depict most of the ducts from the multicellular pedal glands as opening on the propodium and the proximal portion of the metapodium, in the region which remains in contact with the substrate during its pivot stance. In contrast, the foot of D. steinbergae remains in contact with the substrate along its entire length during the period immediately prior to, and during, exit of the larval body from the shell. In this veliger, the ducts from the multicellular pedal glands, although concentrated at the apex of the propodium, are distributed along the entire sole of the foot. This difference in metamorphic behavior between P. sibogae and D. steinbergae, might be related to differences in the shape of the larval shells. P. sibogae possesses an inflated, type 2 shell, while that of D. steinbergae is a spiral, type 1 shell. The shape differences may necessitate the performance of different postures to facilitate detachment of the contracting larval retractor muscle from the inner wall of the shell.

Doridella steinbergae and Adalaria proxima are both detorted, dorid nudibranchs in which the definitive dorsal epidermis is derived from tissue of the mantle fold. However, there are considerable differences in how these processes are effected in the two species. According to Thompson(1958), the retracted mantle fold of A. proxima becomes reflected during the larval stage as a result of differential growth of the cells; reflection of the fold is mechanistically coupled to proliferation and hypertrophy of the mantle fold. Thompson (1958) adds that muscle elements within the perivisceral membrane may aid in the reflection process. Following shell loss in A. proxima, the posterior spread of the reflected mantle fold to form the notum is effected by cell proliferation. Thompson (1958) states that the anus is drawn posteriorly with the migrating mantle tissue and differential growth between the right and left sides of the reflected mantle tissue brings the anus to a medial, posterior position. Subsequent fusion of the two sides of the mantle, behind the anus, places the anal aperture above the notal skirt. Thompson (1958) does not implicate the larval retractor muscle in effecting any of these processes. In D. steinbergae, proliferation and differential growth within the mantle epithelium appears to play no role in either mantle reflection or posterior relocation of the anus. Instead, the larval retractor muscle provides both the direct and indirect mechanism for these processes. As a result, the anal aperture in D. steinbergae moves posteriorly, independent of the peripheral rim of the reflected mantle. Subsequent fusion of the free edge of the mantle fold with the dorsal wall of the anal aperture provides the explanation for the sub-notal position of the anus in this dorid.

The metamorphic process in the aeolid, P. sibogae is not completely comparable to that of D. steinbergae because the definitive dorsal epidermis is derived from epipodial tissue (Bonar and Hadfield, 1974; Bonar, 1976). However, the larval retractor does play a role in reorganizing the viscera in P. sibogae, but only to the extent that it produces axial shifts of the larval body (Bonar and Hadfield, 1974). As in A. proxima, the relocation of the anus in P. sibogae is the result of differential growth, although the anus in the latter species moves posteriorly with the tissues of the foot, rather than with the mantle fold (Bonar and Hadfield, 1974).

In addition to the processes of detorsion and formation of the definitive epidermis, the stomach and left digestive gland of nudibranchs undergo considerable positional change in relation to both each other, and to the cephalopedal mass. In adult dorids, the compact digestive gland occupies most of the ventral portion of the body and the small stomach is located on its mid-dorsal surface. In A. proxima, this arrangement is achieved simply by differential growth between the left digestive gland and the stomach. As described by Thompson (1958), this process begins in the larval stage. As the digestive gland progressively enlarges it extends beneath the stomach. This causes a slight clockwise rotation of the stomach (as viewed anteriorly). At metamorphosis, the rapid growth of the digestive gland beneath the stomach displaces the latter to a mid-dorsal position.

In D. steinbergae, displacement of the stomach by the digestive gland does not occur during the larval phase, and the process which relocates the stomach during metamorphosis is the opposite of that in A. proxima. Instead of the digestive gland moving under the stomach, the stomach moves dorsal to the digestive gland. The dorsal location of the stomach in D. steinbergae is evident in living animals immediately after shell loss. It can be seen as a small patch of white overlying the pigmented digestive gland (Fig. 21f). The rapid attainment of this condition argues against differential growth as the mechanism of displacement. Instead, the initial displacement is effected by contraction of the larval retractor muscle, to which both the stomach and left digestive gland are attached. Differential growth between the stomach and digestive gland accounts for only the completion of this repositioning process.

In adult dorids, the intestine leaves the stomach anteriorly, rather than posteriorly as in the larva. In A. proxima, this condition is achieved by a combination of displacement of the stomach by the digestive gland, and differential growth of various regions of the stomach wall (Thompson, 1958). In D. steinbergae, the condition is achieved by anterior rotation of the stomach by the larval retractor muscle; a process which necessarily reverses the anterior and posterior ends of this organ. Therefore, it is not the intestine which migrates towards the anterior of the stomach, but the stomach itself which inverts.

The results of this study document the process by which the gut of a planktotrophic nudibranch veliger becomes converted to that of the carnivorous, benthic stage. The conversion involves an initial, rapid sloughing of the dorsal stomach cells, and subsequent loss of the cuticle-coated cells of the ventral stomach. Therefore, the adult gut appears to be derived from only the ciliated regions of the ventral stomach which surround the openings of the esophagus and the left digestive gland. Another dramatic change in the gut is the onset of whole-cell phagocytosis by the cells of the digestive gland. In _D_. _steinbergae_, no evidence for this was found in larval stages. Phagocytic activity of particulate material is apparent in the digestive gland of all metamorphic and post-metamorphic stages. Progressive disappearance of the lipoid vesicles from the digestive gland cells during the non-feeding, metamorphic period supports Switzer-Dunlap's (1978) suggestion that these represent stored energy reserves. The lipoid vesicles which accumulate within the stomach cells during larval life are likely metabolized by the digestive gland also, since the stomach cells, following dissociation, are phagocytized by the digestive gland cells. The dense, purple-blue vesicles of the larval digestive gland become replaced in the juvenile by zymogen cells. These are recognized by their content of numerous, small, densely-stained vesicles. This transformation is likely the morphological expression of differing digestive physiologies between the herbivorous, particle-feeding larva, and the carnivorous benthic stage.

Finally, the larval ciliary-mucous mechanisms for the capture, transportation, and sorting of food particles becomes replaced in the juvenile by muscular mechanisms. An extensive, specialized musculature develops in association with the buccal pump and the odontophore, and circular, peristaltic muscles encircle much of the remainder of the digestive tract.

An extraordinary feature of the metamorphosis of the alimentary tract is the short duration of the period in which the redesign of the entire system is accomplished. Within 2 days after shell loss, the transformations which are apparent include: the formation and muscularization of the buccal pump, functional differentiation of the odontophore muscles, differentiation of the salivary glands, formation of the oral lips and growth of their associated glands, loss of the larval components of the stomach, and differentiation of zymogen cells within the digestive gland.

D. GROWTH AND FEEDING OF JUVENILES

Thompson (1964) recognized two basic types of life histories among nudibranchs of the northeastern Atlantic: annuals and seasonals. For each species, the type of life history which is exhibited is correlated with the temporal abundance of the respective prey organism. The results of the present study indicate that D. steinbergae belongs to the category of a seasonal nudibranch. This species passes through a number of generations per year (minimum egg to egg generation period, under laboratory conditions, was 59 days), attains sexual maturity rapidly (22 to 26 days after metamorphosis), spawns throughout adult life, and the field populations are characterized by a broad range of size classes. This type of life history facilitates maximum exploitation of a transient food supply (Thompson, 1964), such as the bryozoan, Membranipora villosa. In the waters surrounding San Juan Island, this bryozoan is abundant only during the period extending from May to October. Electra [Membranipora] crustulenta, the bryozoan food of Doridella obscura, differs from M. villosa in that the former is abundant throughout the year. Consequently, although D. obscura exhibits life history characteristics of a seasonal nudibranch, this species is present throughout the year on colonies of its prey organism (Perron and

Turner, 1977). However, Perron and Turner (1977) found that any one population of E. crustulenta is transient because the colonies eventually succumb to silting. Therefore, these authors have suggested that the term 'opportunistic', rather than 'seasonal', is more appropriate for nudibranchs which feed on a transient prey.

The appearance of the gonadal rudiment in the veliger stage may be an additional adaptation for exploitation of a transient prey organism. Precocious development of the germ cells, as seen in D. steinbergae, may facilitate the rapid attainment of sexual maturity during the benthic stage. Future study of larval development in nudibranchs having a seasonal or opportunistic life history may reveal that this is a widespread phenomenon.

Although the feeding mechanisms of adult nudibranchs have been extensively studied (see reviews by Forrest, 1953; Thompson, 1964), the feeding activities of juveniles requires additional attention. Intraspecific size differences often have important survival consequences for animals. Size refuge from predation and physical stresses are two, frequently cited examples. In addition, the size of predaceous animals will often determine the size class of food available to it. This may result in an ontogenetic change in the composition of the diet. However, in the case of those nudibranchs which have been studied, it is the adult prey which provides the metamorphic cue for the competent veliger. Therefore, the newly metamorphosed juvenile may not have the option to choose a size class of food which it can consume in the same manner exhibited by the larger adults. This problem was first noted by Hadfield (1963) while studying the veliger of *Onchidoris fusca*, a nudibranch species which feeds upon barnacles. Although recently metamorphosed juveniles of this species were not observed, Hadfield (1963) speculated that their size would be considerably smaller than that of recently settled barnacles. Therefore, he suggested that the juvenile nudibranchs might graze upon epibenthic diatoms and ciliates until they become sufficiently large to exploit barnacles. In a study of the life history of *Tritonia hombergi*, Thompson (1962) found that both juveniles and adults feed on the anthozoan, *Alcyonium digitatum*. However, unlike the adults, juveniles cannot ingest the calcareous spicules which are found throughout the prey. Therefore, juveniles restrict their feeding to the superficial tissues of *A. digitatum*. Since the tissue ingested by the young nudibranchs lacks spicules, food manipulation is likely less difficult. As a possible consequence, the radular teeth of the young animals are less complex than those of larger animals (Thompson, 1962).

In their study of *Doridella obscura*, Perron and Turner (1977) state that "Two days after casting their shells, juveniles measured only 300 μm in length and were clearly incapable of feeding on the bryozoans which induced their metamorphosis". Presumably these authors believed the size discrepancy between the predator and the prospective prey was prohibitive. However, juveniles of D. steinbergae are capable of feeding on zooids of M. villosa despite the fact that their notum length is 1/3 to 1/5 that of the length of the bryozoan zooecium. According to Perron and Turner (1977), D. obscura does not begin feeding on E. crustulenta zooids until the animals measure 1 mm. in length. During the interim, the young juveniles feed on diatoms and detritus which are scraped from the surface of the bryozoan.

D. steinbergae does not exhibit this type of diet change during the benthic stage of the life cycle. During the first 2 to 3 days after metamorphosis, the post-larvae often remain at a single spot on the substrate from the onset of metamorphosis until feeding on zooids begins. If juveniles were grazing on diatoms at this time, they would be expected to move over the substrate. Secondly, in histological sections of animals fixed 48 hours after shell loss, the lumina of both the stomach and the digestive gland are completely empty. However, in sections through 3 to 4 day old juveniles, apparent food material is obvious within the lumen of the digestive gland.

From the numerous observations of feeding juveniles and adults, it is evident that M. villosa provides the exclusive stimulus for eliciting a feeding response throughout the benthic life of D. steinbergae. This behavior is facilitated by the functional differentiation of both morphological and neurological components. In neurophysiological studies on Aplysia, it has been found that the feeding reflex (consummatory phase of feeding) is elicited only upon reception of a specific chemical cue from the food species (Kupfermann, 1974). Kriegstein et al. (1974) have examined the development of feeding behavior in laboratory reared juveniles of Aplysia californica. They have shown that the fixed action patterns of both the appetitive and consummatory phases of feeding are fully developed at 3 days after settlement, and these action patterns are cued by the absence or presence, respectively, of the food species, Laurencia pacifica. Therefore, if some species of nudibranchs exhibit an ontogenetic change in their diet (Hadfield, 1963; Perron and Turner, 1977), it might be inferred that a corresponding transformation must occur in the sensory, and possibly the motor, components of the feeding reflex.

Young juveniles of D. steinbergae appear to overcome their size handicap, not by effecting a feeding response to substances other than M. villosa, but by a modification of the feeding mechanism employed by larger individuals. The observations made in this study suggest that the cells of the zooid are first dissociated, and the individual cells are subsequently ingested by the juvenile through a hole it has rasped in the frontal membrane. Ingestion is probably effected by combined action of the buccal pump and the radula. The juvenile possibly injects lytic enzymes into the zooecium to dissociate the zooid tissue. Forrest (1953) has suggested that the salivary glands of some nudibranchs may secrete enzymes, particularly amylases and lipases. If this is the case in D. steinbergae the juveniles may employ these secretions extracorporally.

Observations made during the determinations of size-dependent feeding rate indicate that the activity of juveniles is almost entirely involved in feeding, while adults exhibit a more diversified behavioral repertoire. In addition to feeding, older animals congregate, copulate, and lay egg masses. The expression of these alternate activities, which are related to reproductive functions, likely represent the development of specific neurological circuits. However, these activities may also be facilitated by an increase in feeding efficiency. Neurophysiological studies which have been performed on various opisthobranchs indicate that both the onset of feeding activity and the size of the meal is determined by the bulk of food present in the gut. For example, satiation in Aplysia can be mimicked by injections of non-nutritive material into the gut (Susswein, 1977). It is proposed that in D. steinbergae, the rate at which food can be ingested by young juveniles is such that the animals never become fully satiated. As a result, feeding is continuous. However, in older juveniles and adults, large amounts of food can be ingested rapidly and this results in intermittent periods of satiation. Once the gut has been filled, other adult behaviors, particularly those involved in reproductive functions, may be expressed. Davis et al. (1974) have shown that in the notaspidean, Pleurobranchia, activities related to feeding are dominant; in unsatiated animals, feeding behaviors tend to suppress other behavioral reflexes.

It is further suggested that in young juveniles of D. steinbergae, procurement of food is limiting to metabolic rate, while in older juveniles, digestive processes are the limiting factor (assumming that other factors, such as temperature and food abundance are equal). Therefore, it is interesting to note that the onset of the period of rapid juvenile growth (at 10 to 14 days after metamorphosis) coorelates with the onset of the 'adult' mode of feeding (at approximately 13 days after metamorphosis).

TABLES AND FIGURES

Table 1: Timetable of Embryogenesis in Doridella steinbergae (12 - 15°C).

Developmental Events	Time
oviposition	0
1st polar body	2 hours, 25 min.
2nd polar body	4 hours, 30 min.
1st cleavage	8 hours, 25 min.
2nd cleavage	11 hours, 20 min.
3rd cleavage	14 hours
4th cleavage	17 hours
appearance of blastopore	2 days
closure of blastopore	late 2nd day
first sign of cilia	3 days
velar rudiment	3 1/2 days
foot rudiment	3 1/2 days
statocysts	late 3rd day
shell secretion	4 1/2 days
velar ridge	5 days
metachronism	5 1/2 days
ability to stop cilia simultaneously	5 1/2 days
perivisceral space	5 1/2 days
subvelar ridge	5 1/2 days
velar lobes become moveable	6 1/2 days
hatching	7 1/2 to 8 days

Table 2: Induction of Metamorphosis in *Doridella steinbergae*

Experiment Number: 1

Substrate Provided	Age of Larvae (days)	Number of Larvae Introduced	No. Metamorphosed	Latency Period (hours)
M. villosa on *L. saccharina*	28	15	9	1 to 18
L. saccharina	28	15	0	-
M. villosa on glass slide	28	15	9	0.5 to 11.5
glass slide	28	15	0	-

Experiment Number: 2

Substrate Provided	Age of Larvae (days)	Number of Larvae Introduced	No. Metamorphosed	Latency Period (hours)
M. villosa on *L. saccharina*	31	15	10	0.5 to >24 <36
L. saccharina	31	15	0	-
M. villosa on glass slide	31	15	8	2 to 10

Table 3: Effect of Experimental Manipulations of the Induction Substrate

Substrate Provided	Age of Larvae (days)	Number of Larvae Introduced	No. Metamorphosed
Bowl 1: Intact colony of M. villosa	31	15	10
Bowl 2: Periphery of colony only	31	15	4
Bowl 3: Center of colony only	31	15	2
Bowl 4: Periphery of colony vs. center of colony	31	15	periphery:4 center: 0

Table 4: Data on Juvenile Feeding

Age at Time of Attack on Zooid (days)	Size (mm)	Time Period for Consumption (hours)	Amount of Zooid Consumed	Comments
2.25	0.22	40	incomplete	1st zooid consumed during benthic life
3.00	0.22	37	incomplete	
?	0.20	37	incomplete	
2.33	0.22	43	complete	
4.00	0.28	39	incomplete	2nd zooid consumed during benthic life
4.66	0.28	39	complete	
4.00	0.28	36	complete	
9.00	0.49	11	incomplete	
10.00	0.47	12	complete	
10.00	0.53	15	complete	
13.00	1.07	2.25	complete	tissue of zooid is not dissociated prior to ingestion
13.00	1.04	1.25	complete	
13.00	1.04	1.75	complete	
13.00	1.12	1.50	complete	

Figure 1: Adult Doridella steinbergae on its natural substrate of Membranipora villosa encrusted on brown algae

a. Note the absence of branchiae on the dorsal surface of the notum. Scale bar = 3 mm

b. The same animal, illustrating crypsis. Scale bar = 4 mm

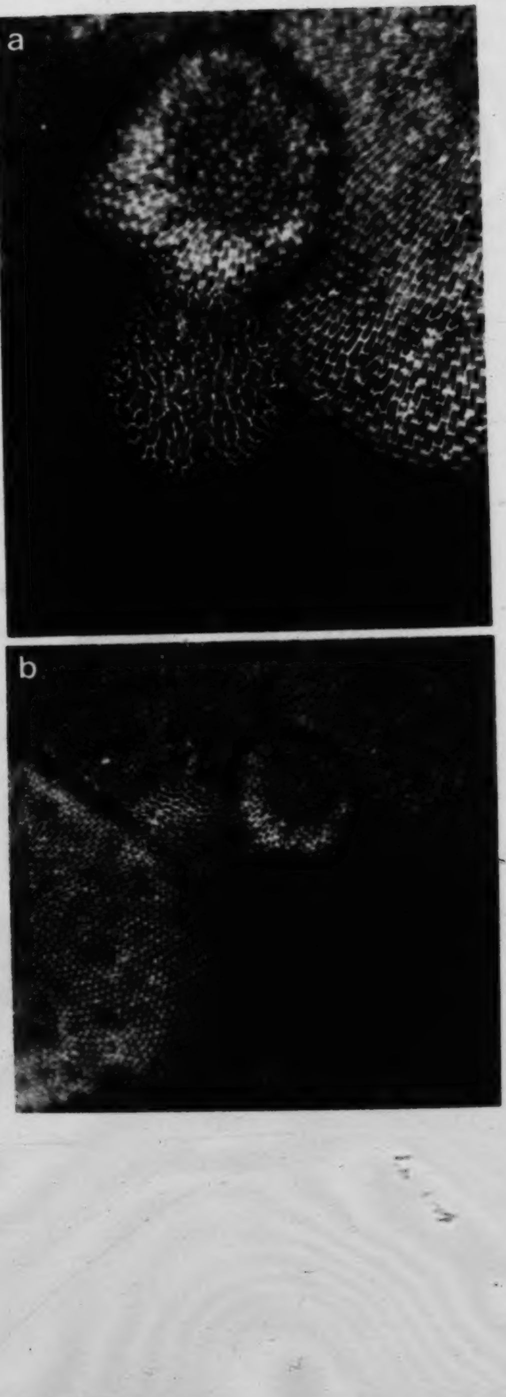

a
b

Figure 2: Development of the veliger of *D. steinbergae* during the first half of larval life (veligers reared according to culture technique I).

a. Right, lateral view of a newly hatched veliger (stage I) The larval shell consists of one-half whorl and the left digestive gland is unpigmented. Scale bar = 25 µm

b. Ventral view of a newly hatched veliger (stage I). The arrow indicates the bulge in the left digestive gland produced by the large, undifferentiated cells. Scale bar = 25 µm

c. Right, lateral view of a 2 day old veliger (stage I). Note the growth of the larval shell and the pigmentation of the left digestive gland. The arrow indicates the undifferentiated cells of the left digestive gland which do not acquire algal pigments after feeding has begun. Slender fibers of the mantle retractor muscles can be seen extending from the perivisceral membrane to the mantle fold. Scale bar = 25 µm

d. Right, lateral view of 5 day old veliger (stage I). Note the size difference between the right and left digestive gland. A ridge in the stomach wall demarcates the ventral and dorsal portions. The larval kidney vesicle is a large spherical structure located adjacent to the terminal intestine. Scale bar = 25 µm

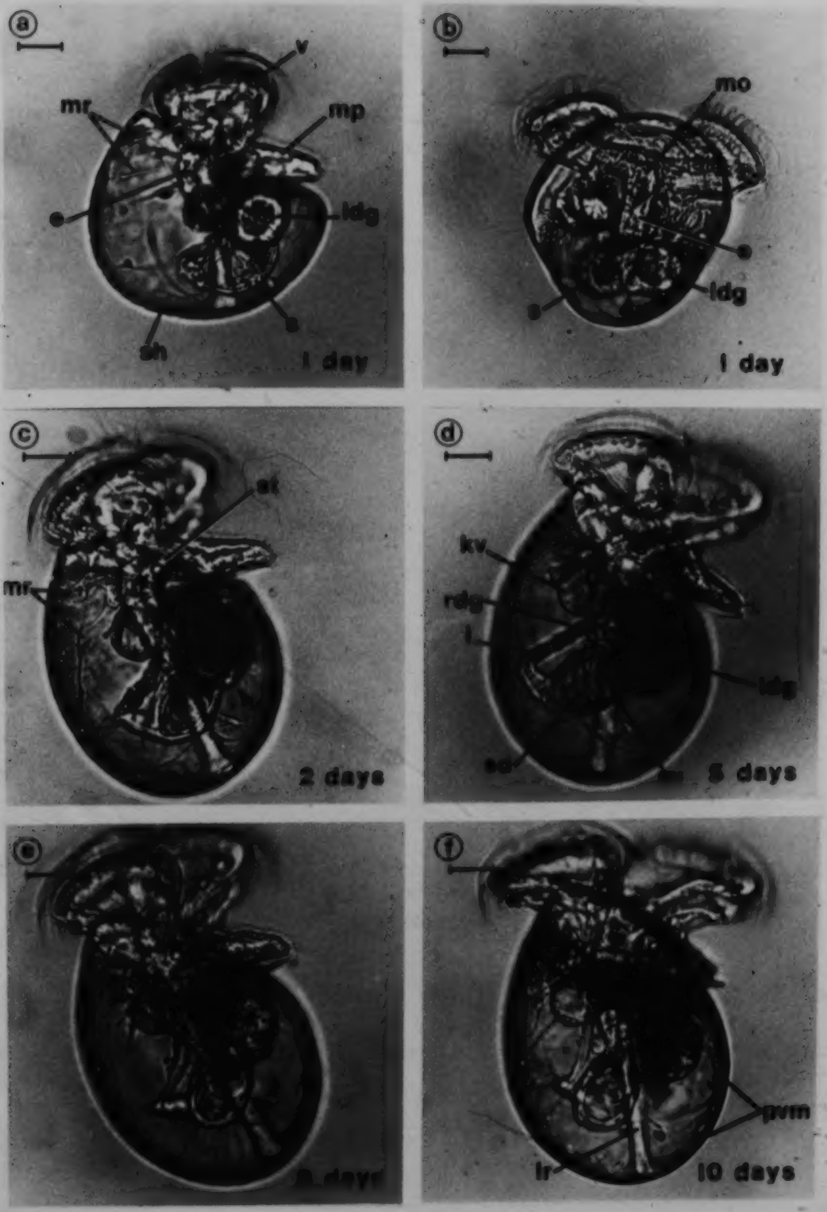
e. Right, lateral view of 8 day old veliger (stage I). Scale bar = 25 µm

f. Ventro-lateral view of 10 day old veliger (stage I). The perivisceral membrane can be seen as a thin membrane lining the inner wall of the larval shell. Scale bar = 25 µm

Legend:

e - esophagus
i - intestine
kv - larval kidney vesicle
ldg - left digestive gland
lr - larval retractor muscle
mo - mouth
mp - metapodium
mr - mantle retractor muscle
pvm - perivisceral membrane
rdg - right digestive gland

s - stomach
sd - dorsal stomach
sh - larval shell
st - statocyst
sv - ventral stomach
v - velum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1 day
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1 day
c
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mr
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d
kv
rdg
l
ldg
sd
5 days
e
8 days
f
pvm
lr
10 days

Figure 3: Development of the veliger of D. steinbergae during the last half of larval life (veligers reared according to culture technique I).

a. Right, lateral view of 14 day old veliger (late stage I). Scale bar = 25 μm

b. Right, lateral view of a 16 day old veliger (onset of stage II). The mantle fold has just begun to retract from the aperture of the larval shell. Note the mantle retractor muscles extending from the perivisceral membrane to the partially retracted mantle fold. Scale bar = 25 μm

c. Right, lateral view of a 16 day old veliger (stage II). The mantle is fully retracted. The larval kidney vesicle has been drawn posteriorly with the mantle fold. Note the small eyespots. Scale bar = 25 μm

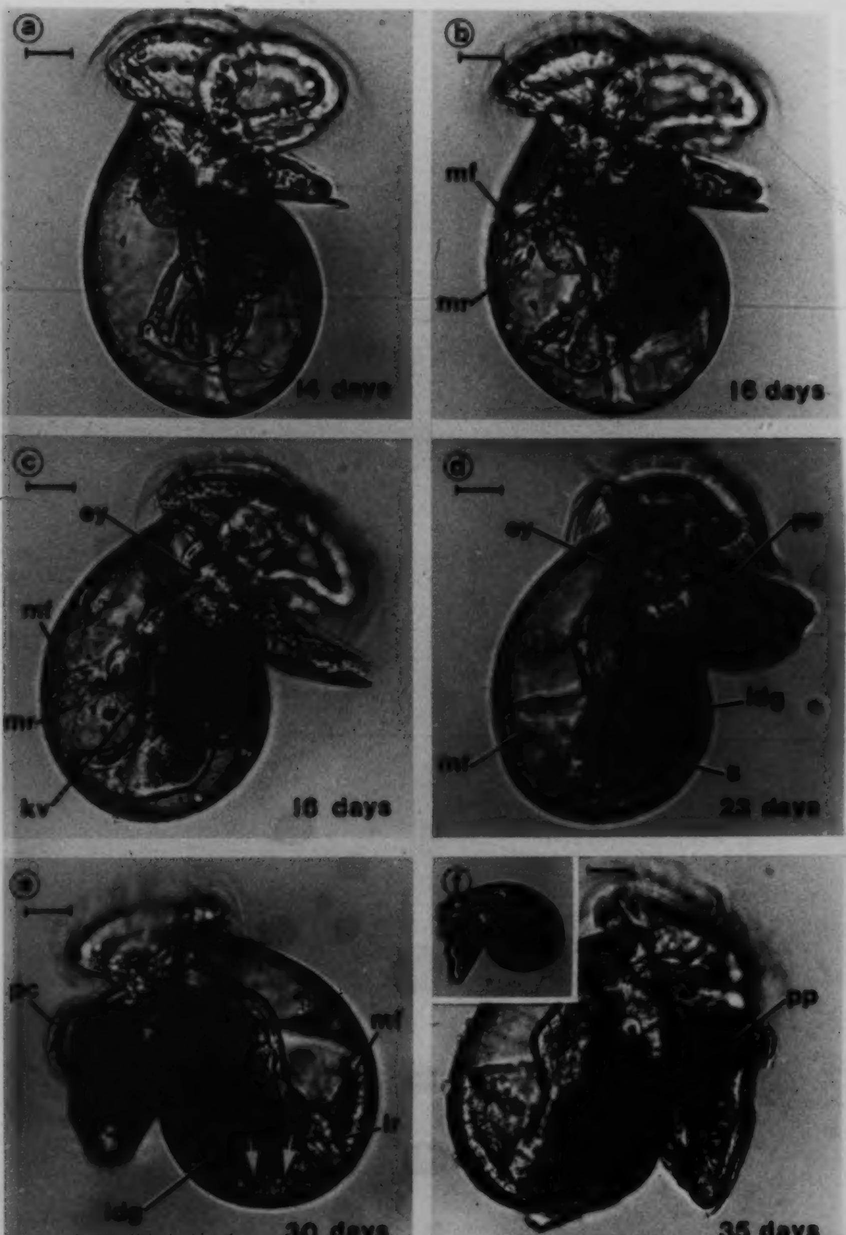
d. Right, lateral view of a 23 day old veliger (stage III). The swelling on the proximal portion of the foot is the prospective propodium. Note the thickening of the mantle fold tissue. Scale bar = 25 μm

e. Left, lateral view of a 30 day old veliger (late stage III). Note the further enlargement of the propodium. The left digestive gland has become large, and the arrows indicate the lipoid vesicles within this organ. The mantle fold has undergone further hypertrophy. Scale bar = 25 μm

f. Lateral views of 35 day old veligers which are competent to metamorphose (stage IV). Note full development of the propodium. Inset shows partially retracted veliger illustrating the action of the subepidermal pedal muscles in contorting the sole of the foot. Scale bar = 25 μm

Legend:

ey - eyespot
i - intestine
kv - larval kidney vesicle
ldg - left digestive gland
lr - larval retractor muscle
mf - mantle fold
mr - mantle retractor muscle
pc - pedal ciliation
pp - propodium
s - stomach


a
14 days
b
mf
mr
16 days
c
ey
mf
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kv
18 days
d
ey
pp
ldg
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s
23 days
e
pc
mf
lr
ldg
30 days
f
pp
35 days

Figure 4: Growth of the larvae of D. steinbergae as determined by measurements of two parameters: the left digestive gland and the larval shell. The onset of each of the four larval stages is indicated. Veligers were reared according to culture technique II.

a. Growth of the left digestive gland. Each point on the graph represents the mean value of 10 larvae measured. The digestive gland index was computed as follows:

$$\text{digestive gland index} = \frac{d^{max} \times d^{min}}{100}$$

where, d^{max} = maximum diameter of the gland (um)

d^{min} = minimum diameter of the gland (um)

b. Growth of the larval shell. Each point on the graph represents the mean of 10 larvae measured. The shell depth dimension was measured according to Hurst (1967).

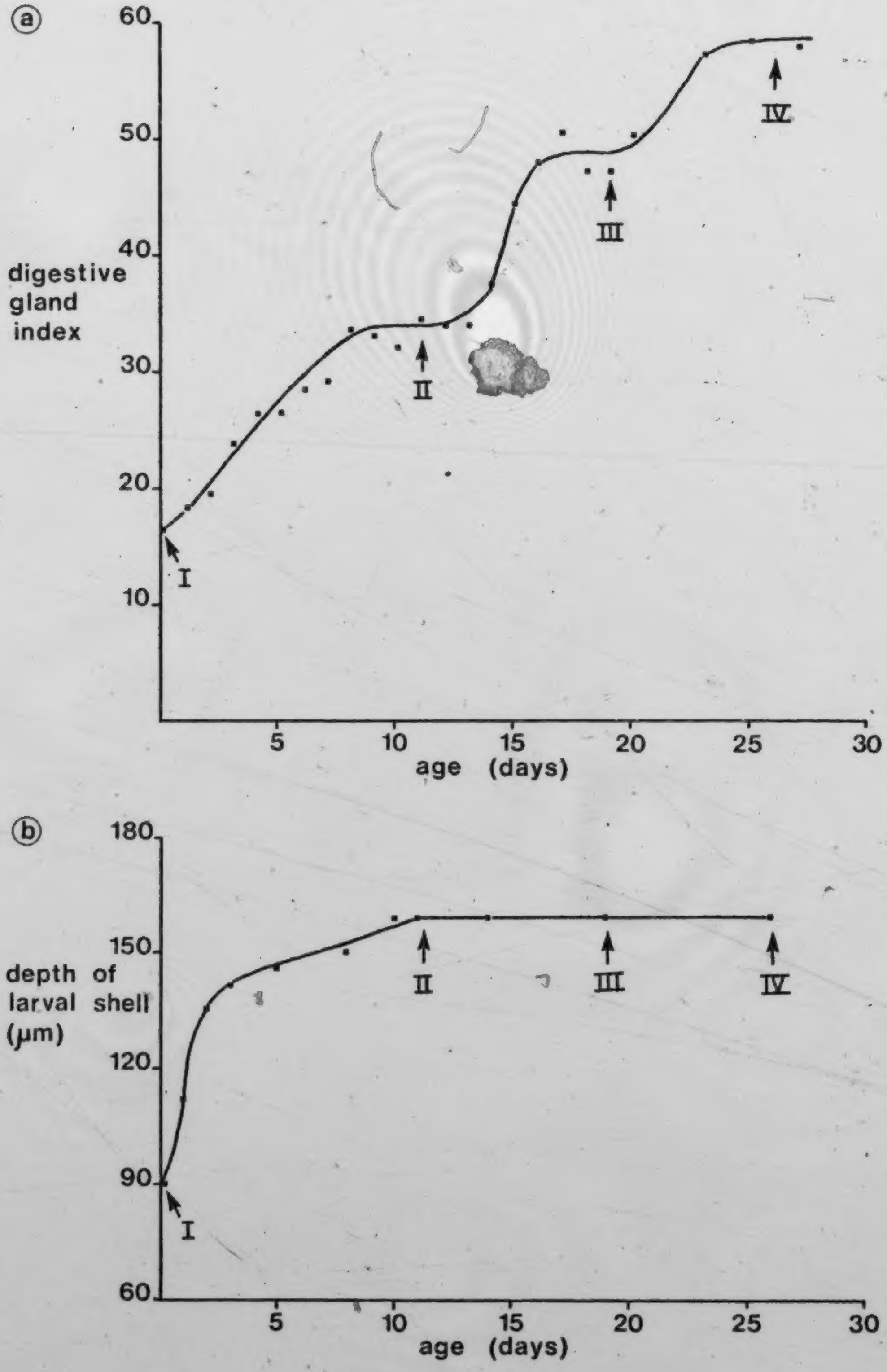

ⓐ
digestive gland index
60
50
40
30
20
10
5
10
15
20
25
30
age (days)
I
II
III
IV
ⓑ
depth of larval shell (μm)
180
150
120
90
60
5
10
15
20
25
30
age (days)
I
II
III
IV

Figure 5: Development of the larval foot

a. Sagittal section of the foot of a newly hatched veliger (stage I). The section passes through the pedal groove. Scale bar = 5 μm

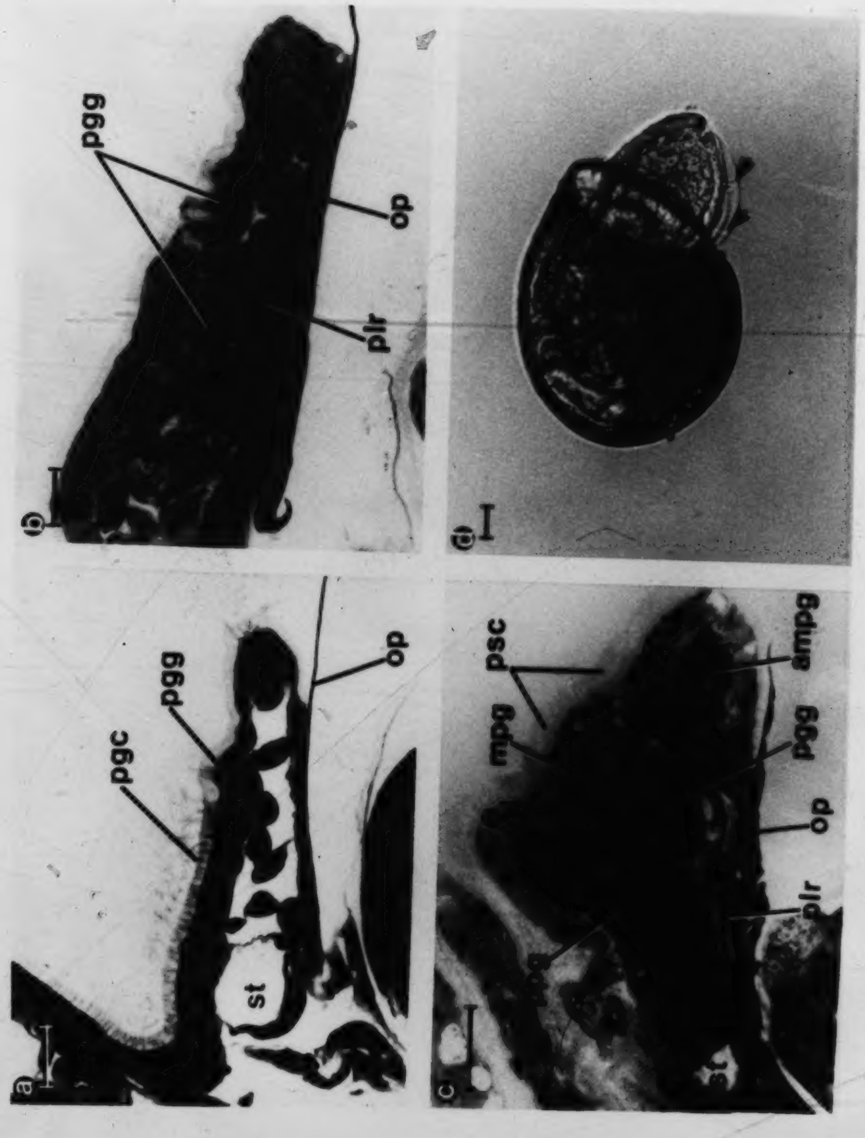
b. Sagittal section of the foot at stage II. Note the enlargement of the pedal groove gland. Scale bar = 5 μm

c. Sagittal section (slightly oblique) of the foot at stage IV. The propodium has developed by proliferation of the pedal epithelium and the differentiation of the large propodial and metapodial glands. Note the dense ciliation which covers the sole of the foot. Scale bar = 20 μm

d. Photomicrograph of a partially retracted veliger at stage III. Arrows indicate the stiff tufts of cilia which arise from the sides of the foot and extend beyond the operculum. Scale bar = 20 μm

Legend:

ampg - accessory metapodial gland
mpg - metapodial gland
op - operculum
pgc - pedal groove ciliated tract
pgg - pedal groove gland
plr - pedal branch of the larval retractor muscle
ppg - propodial gland
psc - pedal sole ciliation
st -statocyst


a
pgc
pgg
op
st
b
pgg
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plr
c
psc
mpg
ampg
pgg
op
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st
d

Figure 6: Development of the larval foot
Cross-sections through the proximal region of the foot in:

a. Stage I. The cilia are restricted to a tract running down the pedal groove and two lateral tracts. Scale bar = 10 µm

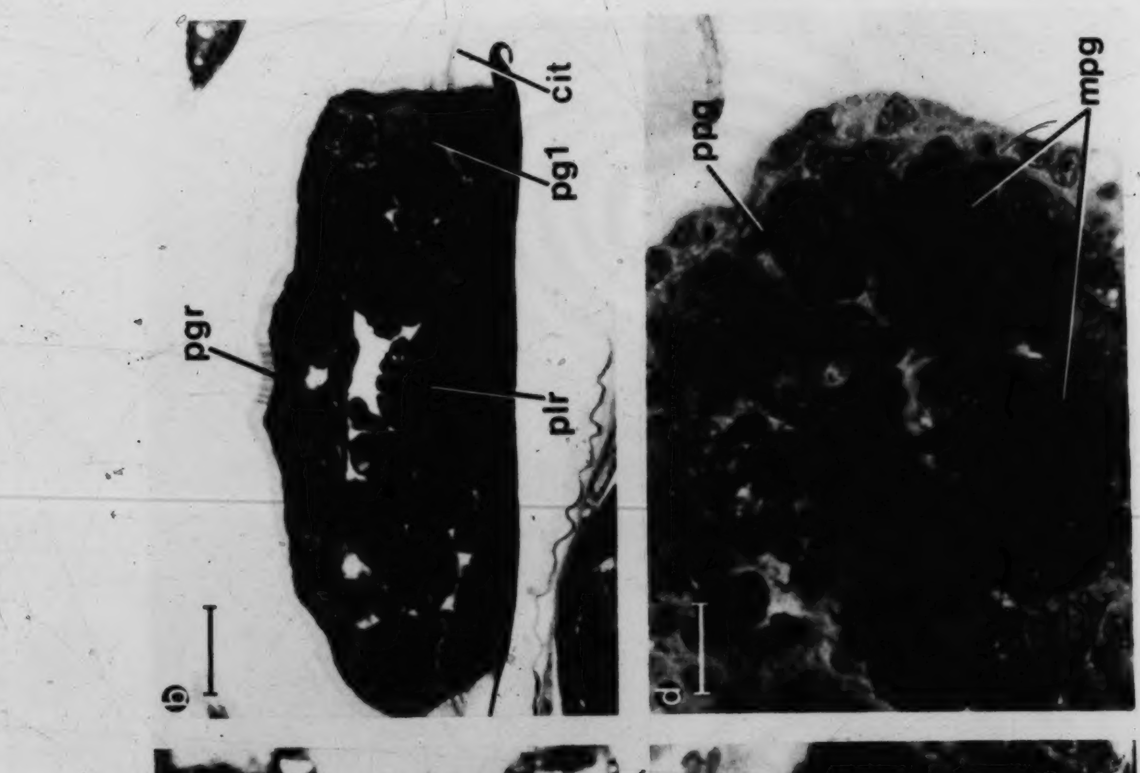
b. Stage II. Note the ciliary tuft on the lateral side of the foot. Scale bar = 10 µm

c. Stage III. Note accumulation of undifferentiated cells within this region of the foot. Scale bar = 10 um

d. Stage IV. Note the differentiation of the propodial and metapodial glands. Scale bar = 10 µm

Legend:

cit - ciliary tuft
mpg - metapodial gland
op - operculum
pg1 - type 1 unicellular pedal gland
pg2 - type 2 unicellular pedal gland
pgr - pedal groove
plr - pedal branch of the larval retractor muscle
ppg - propodial gland


a
pg1
pgr
op
b
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cit
pg1
plr
c
pg2
d
ppg
mpg

Figure 7: Development of the distal region of the larval esophagus

a. Frontal section of a newly hatched veliger (stage I) passing through the mouth. Scale bar = 5 µm

b. Frontal section of a mid-stage I veliger passing through the distal esophagus and the mouth. Note the uniformly cuboidal epithelium of the esophagus. Scale bar = 10 µm

c. Frontal section of a stage II veliger passing through the distal esophagus. Note the hypertrophy of the cells forming the ventral wall of the esophagus (prospective radular rudiment). Scale bar = 10 µm

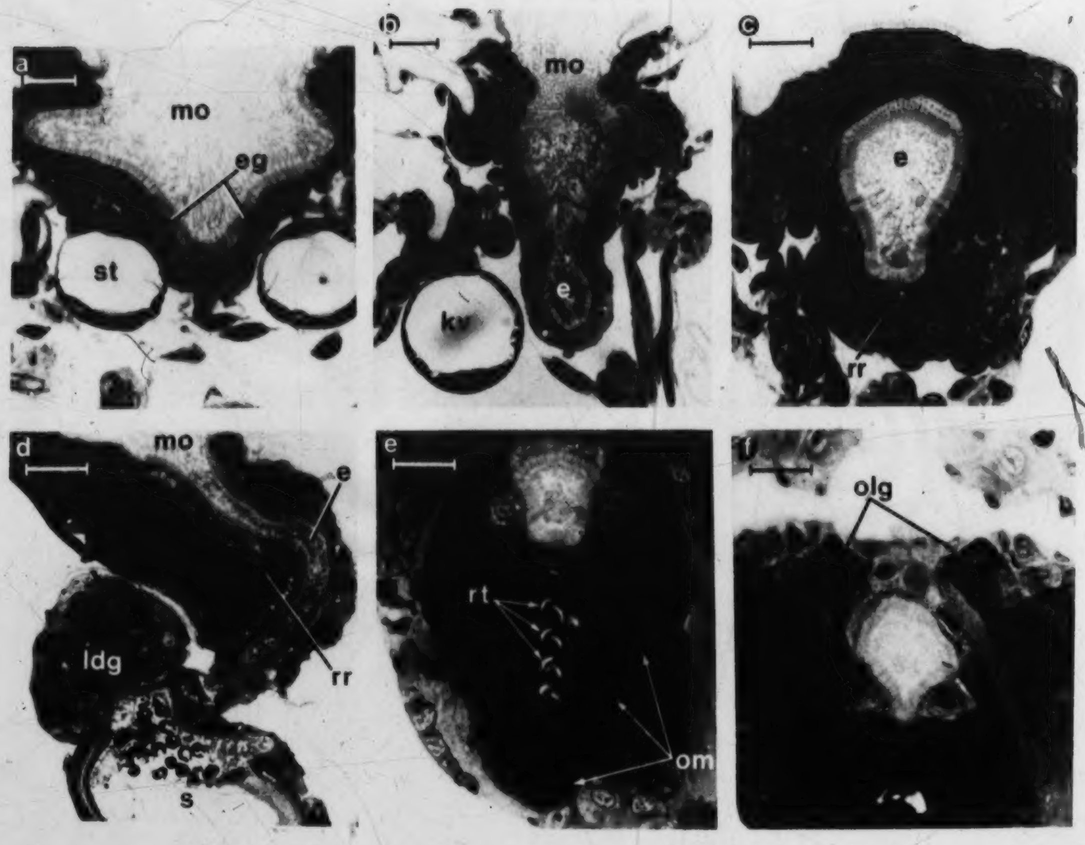
d. Sagittal section of a stage III veliger showing the rudiment of the radular sac evaginating from the ventral wall of the esophagus. Scale bar = 20 µm

e. Frontal section of a stage IV veliger showing the radular teeth and the rudimentary odontophore musculature. Scale bar = 10 µm

f. Frontal section through distal esophagus of a stage IV veliger showing the rudiments of the oral lip glands. Scale bar = 10 µm

Legend:

e - esophagus
eg - esophageal glands
kv - larval kidney vesicle
ldg - left digestive gland
mo - mouth
olg - oral lip gland
om - rudimentary odontophore muscles
rr - rudiment of the radular sac
rt - radular teeth
s - stomach
st -statocyst


a
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e
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om
f
olg

Figure 8: Structure of the larval alimentary tract

a. Sagittal section through a newly hatched veliger (stage I) showing the morphological differentiation of the stomach into dorsal and ventral portions. Note the entry of the esophagus into the ventral chamber. Scale bar = 20 μm

b. Sagittal section through a stage III veliger illustrating that the organization and histological characteristics of the gut are basically unchanged from those of stage I. An exception is the appearance of lipoid vesicles in some of the stomach cells (indicated by arrows). Scale bar = 20 μm

c. Frontal section through a stage II veliger showing entry of left digestive gland into the ventral stomach. Arrows indicate unicellular gland cells which are found only in cuticle-coated regions of the ventral stomach. Note the algal cells in the lumina of the left digestive gland and the stomach. This section also shows the mucous rod which continued through the dorsal stomach in subsequent serial sections of this larva. Scale bar = 20 μm

d. Cross section of the ventral stomach showing the refractile rods embedded in the cuticle. Scale bar = 10 μm

e. Cross section of the dorsal stomach. Note the band of densely packed cilia and the intestinal groove on the upper wall. Scale bar = 10 μm

Legend:

ac - algal cells
cib - ciliated band
cu - cuticle
e - esophagus
i - intestine
ig - intestinal groove
ldg - left digestive gland
mr - mucous rod
pvm - perivisceral membrane
rer - refractile rods
rdg - right digestive gland
sd - dorsal stomach
sv - ventral stomach

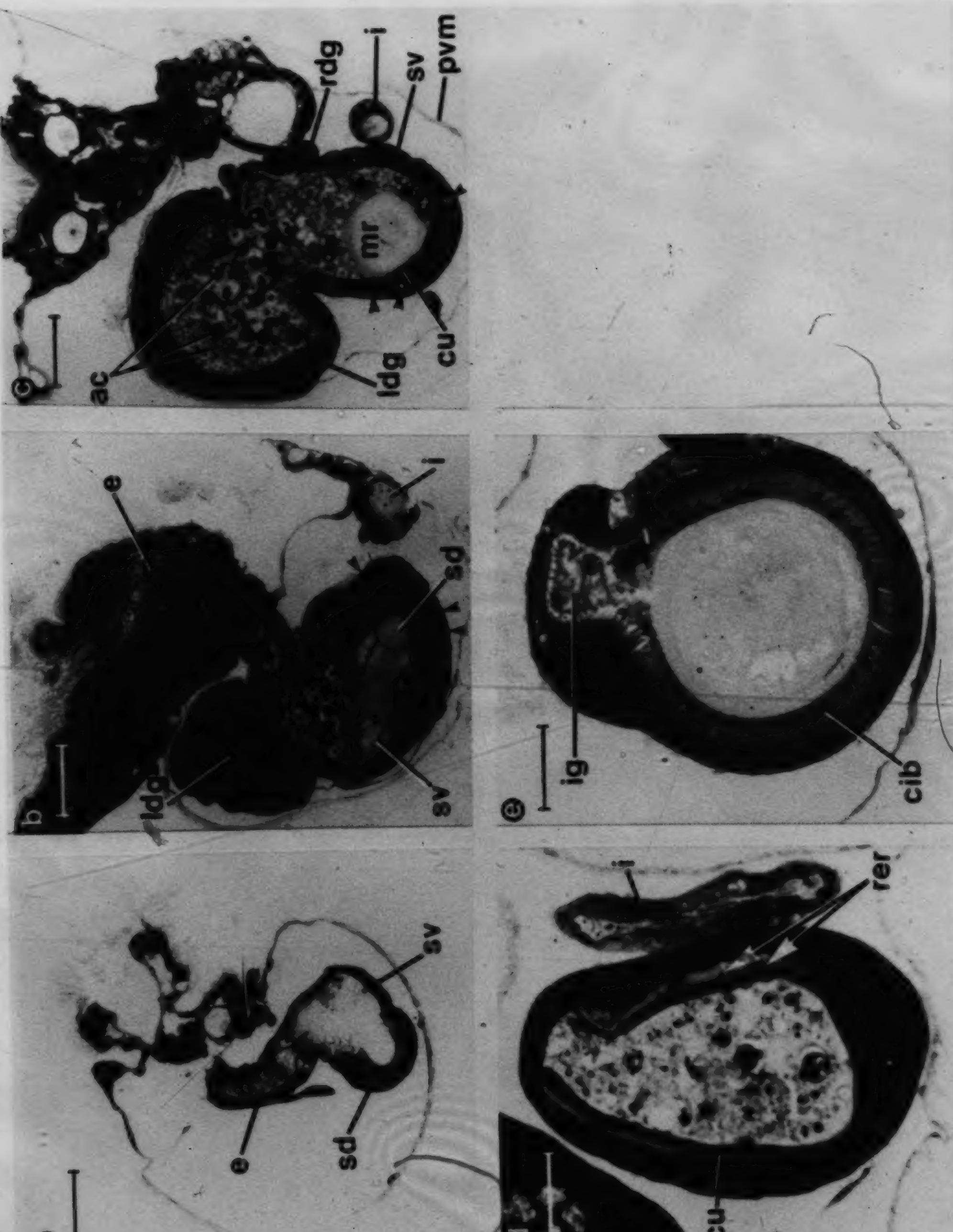
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Figure 9: Developmental changes of the left digestive gland

a. Left digestive gland of a newly hatched veliger (stage I). The gland is composed of sparsely ciliated digestive cells and several large cells with a dense, homogeneous cytoplasm and prominent nuclei and nucleoli (dg2). Note the presence of spherical, densely staining vesicles in some of the digestive cells. Scale bar = 10 μm

b. Left digestive gland of a stage II veliger. The arrow indicates one of the densely staining, spherical vesicles within the lumen of the gland. Other densely staining vesicles, and vacuoles containing a flocculent material are located within the digestive cells. Scale bar = 10 μm

c. Left digestive gland of a stage III veliger. Note the enlargement of the digestive cells and the presence of lipoid vesicles within some of the cells. Scale bar = 10 μm

d. Left digestive gland of a stage IV veliger. The digestive cells are large and pyramidal in shape and contain lipoid, flocculent, and densely staining vesicles. Scale bar = 10 μm

Legend:

ag - algal cells
dg2 - type 2 digestive gland cells
dv - densely staining vesicles
fv - flocculent vesicles
lv - lipoid vesicles

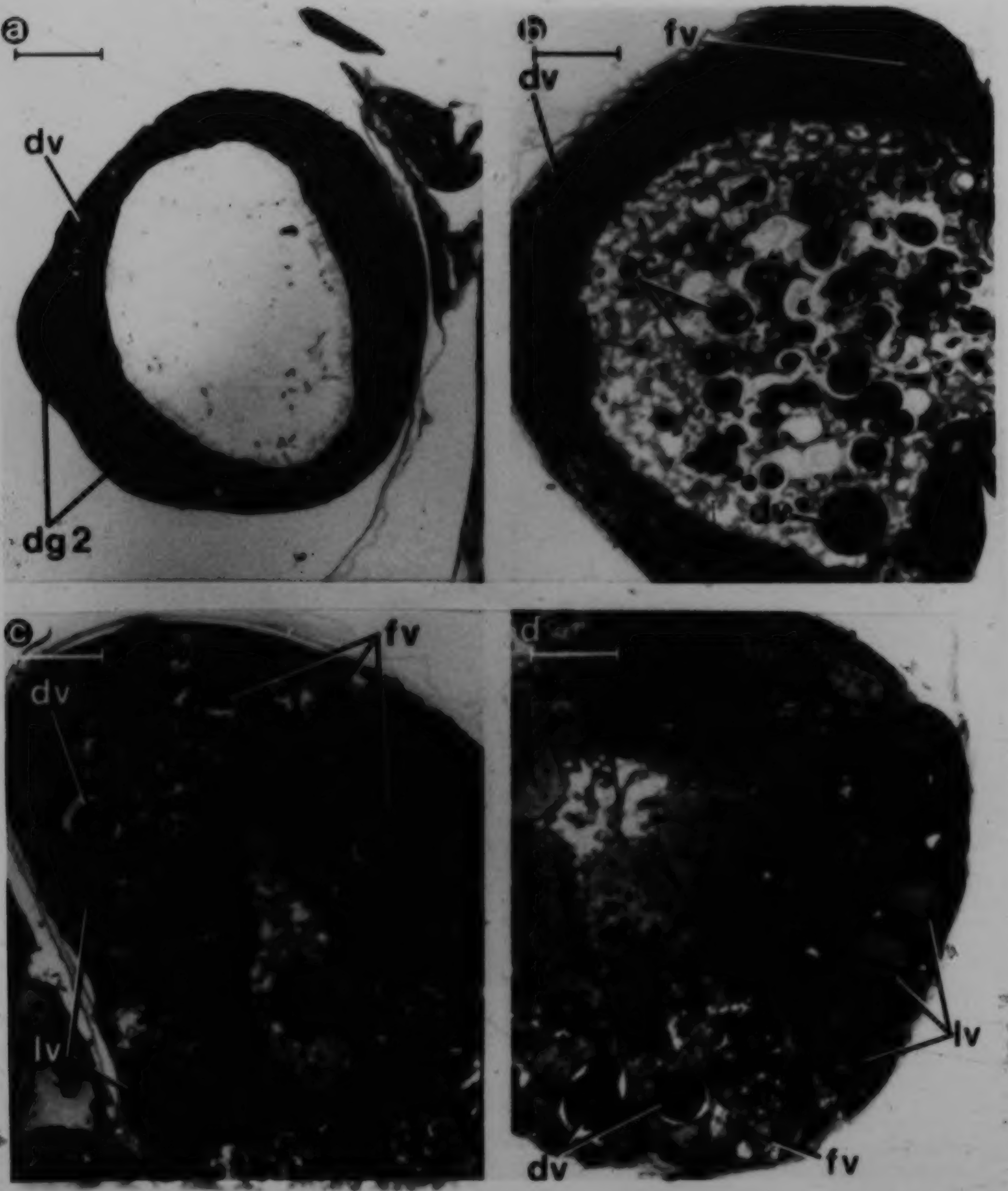
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Figure 10: Nervous system of stage I and stage II veligers

a. Frontal section of a newly hatched veliger (stage I) showing the cerebral ganglia located on each side of the distal esophagus. Scale bar = 20 μm

b. Frontal section of a newly hatched veliger (stage I) showing the statocysts. The pedal ganglia, which eventually develop in close proximity to the statocysts are not present at this stage of development. Note that the depth of the shallow mantle cavity is equal on both sides of the newly hatched veliger. Scale bar = 20 μm

c. Frontal section of a stage II veliger passing through the cerebral ganglia and showing the cerebral commissure. Scale bar = 20 μm

d. Frontal section of a stage II veliger passing through the statocysts. The pedal ganglia have developed on the antero-ventral faces of the statocysts. Scale bar = 20 μm

e. Frontal section of a stage II veliger showing one member of the pair of pleural ganglia and the invaginating rudiments of the optic ganglia. Scale bar = 20 μm

Legend:

cc - cerebral commissure
cg - cerebral ganglion
e - esophagus
kv - larval kidney vesicle
ldg - left digestive gland
lr - larval retractor muscle
mc - mantle cavity
mf - mantle fold
og - optic ganglion
plg - pleural ganglion
pg - pedal ganglion
st - statocyst

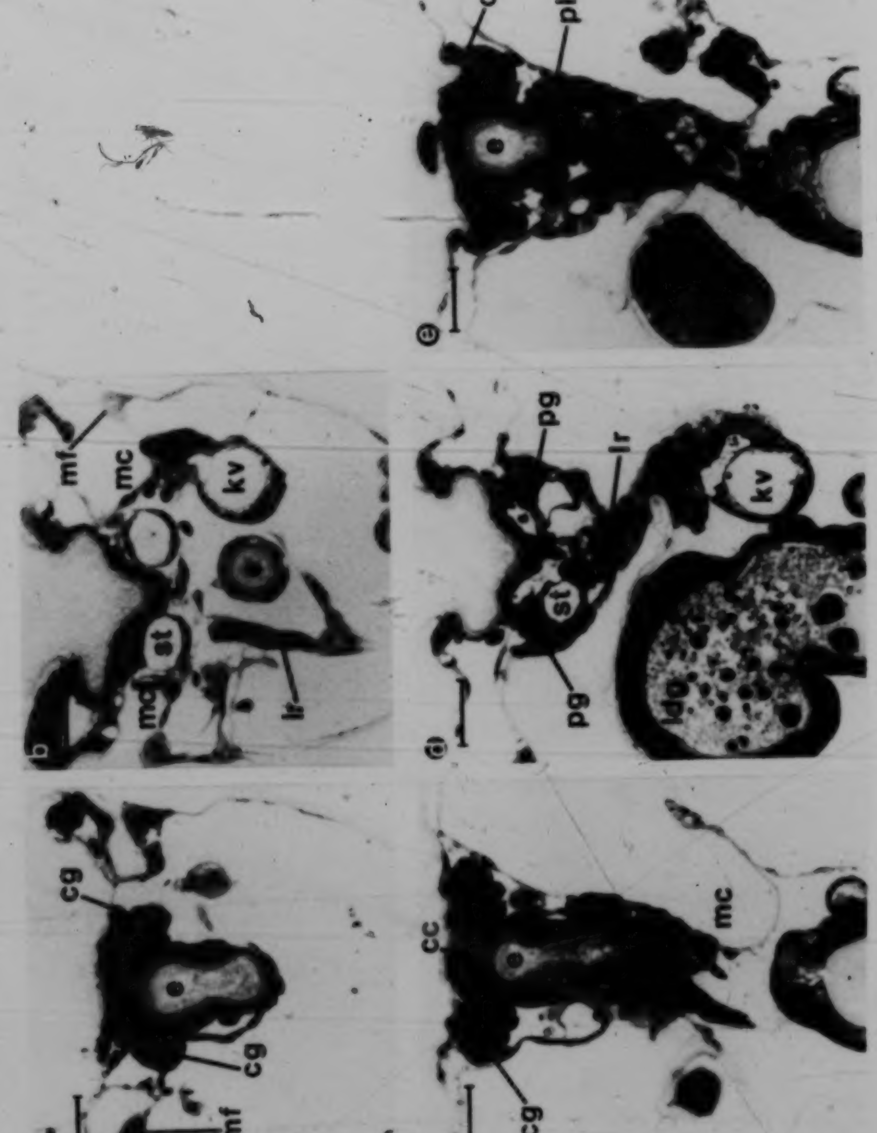
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Figure 11: Nervous system of stage III and stage IV veligers

a. Cross section of a stage III veliger. Note the enlargement of the cerebral ganglia. The eyespots are fully differentiated; the pigmented cells and the lens can be seen in this section. Scale bar = 20 µm

b. Cross section of a stage III veliger. Note the marked enlargement of the pedal ganglia and their differentiation into cortexes and medullae. Scale bar = 20 µm

c. Cross section of a stage III veliger showing the pleural and buccal ganglia. The latter pair is connected by the buccal commissure which runs between the esophagus and the rudiment of the radular sac. Scale bar = 20 µm

d. Frontal section of a competent veliger (stage IV) showing the pleural ganglia and the large cerebral ganglia. Scale bar = 20 µm

e. Frontal section of a competent veliger (stage IV) showing the further enlargement of the pedal ganglia. Scale bar = 20 µm

f. Frontal section of a competent veliger (stage IV) showing the optic ganglia located immediately beneath the eyespots, and the further enlargement of the buccal ganglia. Scale bar = 20 µm

Legend:

bc - buccal commissure
bg - buccal ganglion
cc - cerebral commissure
cg - cerebral ganglion
ey - eyespot
gr - gonadal rudiment
ldg - left digestive gland
lr - larval retractor muscle
mc - mantle cavity
og - optic ganglion
op - operculum
pc - pedal commissure
plg - pleural ganglion
pg - pedal ganglion
rr - rudiment of the radular sac
st - statocyst

L

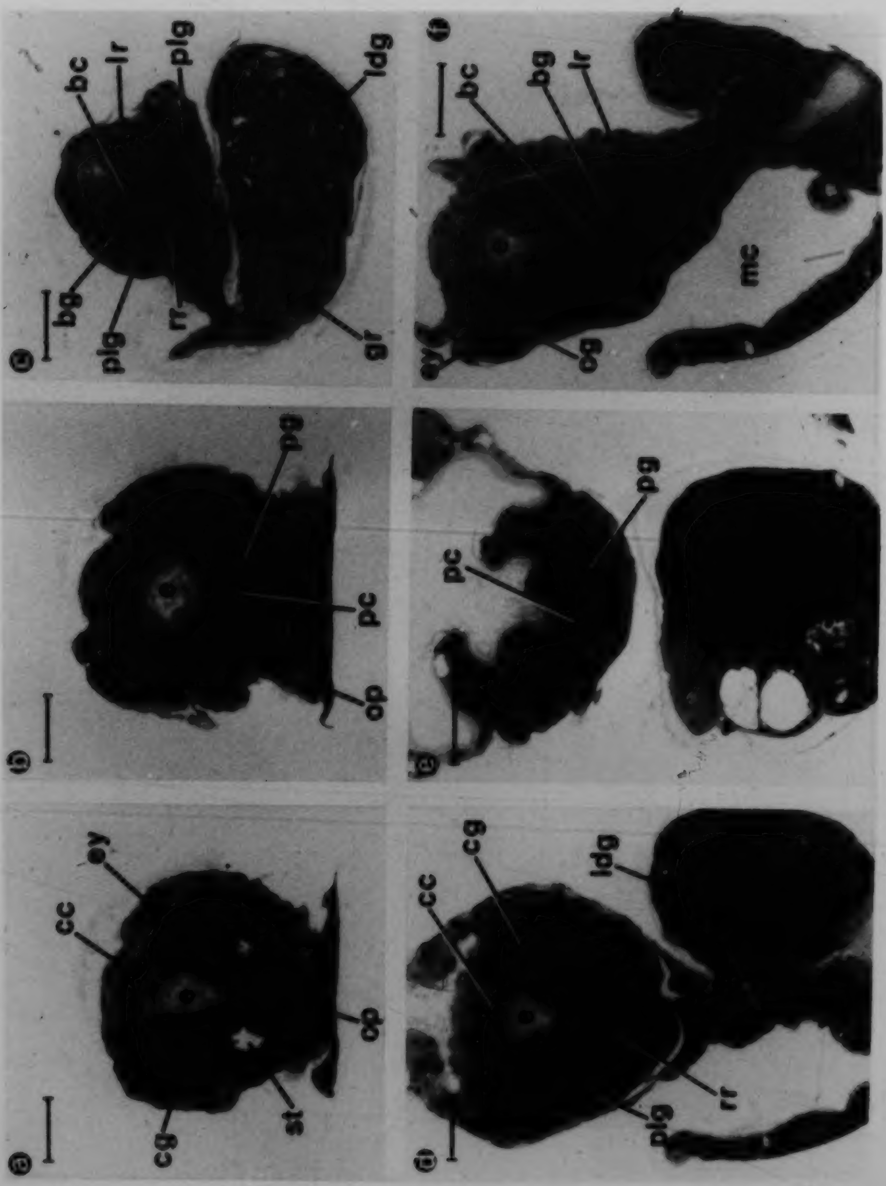
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Figure 12: Larval kidney complex

a. Sagittal section of a newly hatched veliger (stage I) passing through 3 cell types of the larval kidney complex. Scale bar = 10 µm

b. Cross section of a mid-stage I veliger. The section passes through the distal portions of the kidney complex and illustrates the relative positions of the kidney cell types. Scale bar = 10 µm

c. Sagittal section of a stage III veliger. Note the enlargement of the vacuoles within the larval kidney vesicle and the type a cells. This section also shows the type c cell. Scale bar = 10 µm

d. Sagittal section of a competent veliger (stage IV) passing through all 4 cell types of the larval kidney complex. Scale bar = 10 µm

e. Sagittal section through extreme right side of a competent veliger (stage IV) showing the rudiment of the adult kidney. Scale bar = 10 µm

Legend:

a - type a larval kidney cell
akr - adult kidney rudiment
an - anus
b - type b larval kidney cell
c - type c larval kidney cell
d - type d larval kidney cell
e - esophagus
i - intestine
kv - larval kidney vesicle
mf - mantle fold
pvm - perivisceral membrane
s - stomach
yg - yolk granule

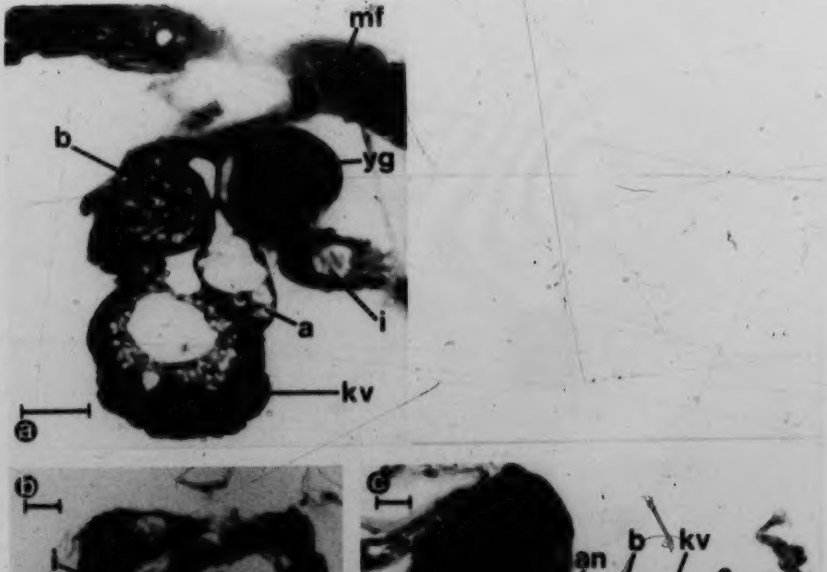

mf
b
yg
a
i
kv
a
b
i
a
kv
b
pvm
v
r
l
d
c
an
b
kv
c
mf
a
d
d
b
a
kv
c
mf
s
e
d
kv
akr

Figure 13: Development of the gonadal rudiment

a. Frontal section of a newly hatched veliger (stage I). The presumptive gonadal rudiment is a group of cells adjacent to the distal end of the intestine. These cells encompass a large granule of apparent yolk. Scale bar = 20 μm

b. Frontal section of a stage II veliger. The primary germ cells of the gonadal rudiment are recognizable at this stage. Scale bar = 20 μm

c. High magnification of the primary germ cells at stage III. The cytoplasm contains numerous, small granules and the nuclei and nucleoli are prominent. Scale bar = 10 μm

d. Frontal section of a competent veliger (stage IV) showing the enlargement of the gonadal rudiment and the position of the rudiment relative to the left digestive gland. Scale bar = 20 μm

Legend:

gr - gonadal rudiment
i - intestine
kv - larval kidney vesicle
ldg - left digestive gland
mf - mantle fold
mp - metapodium
s - stomach
yg - yolk granule

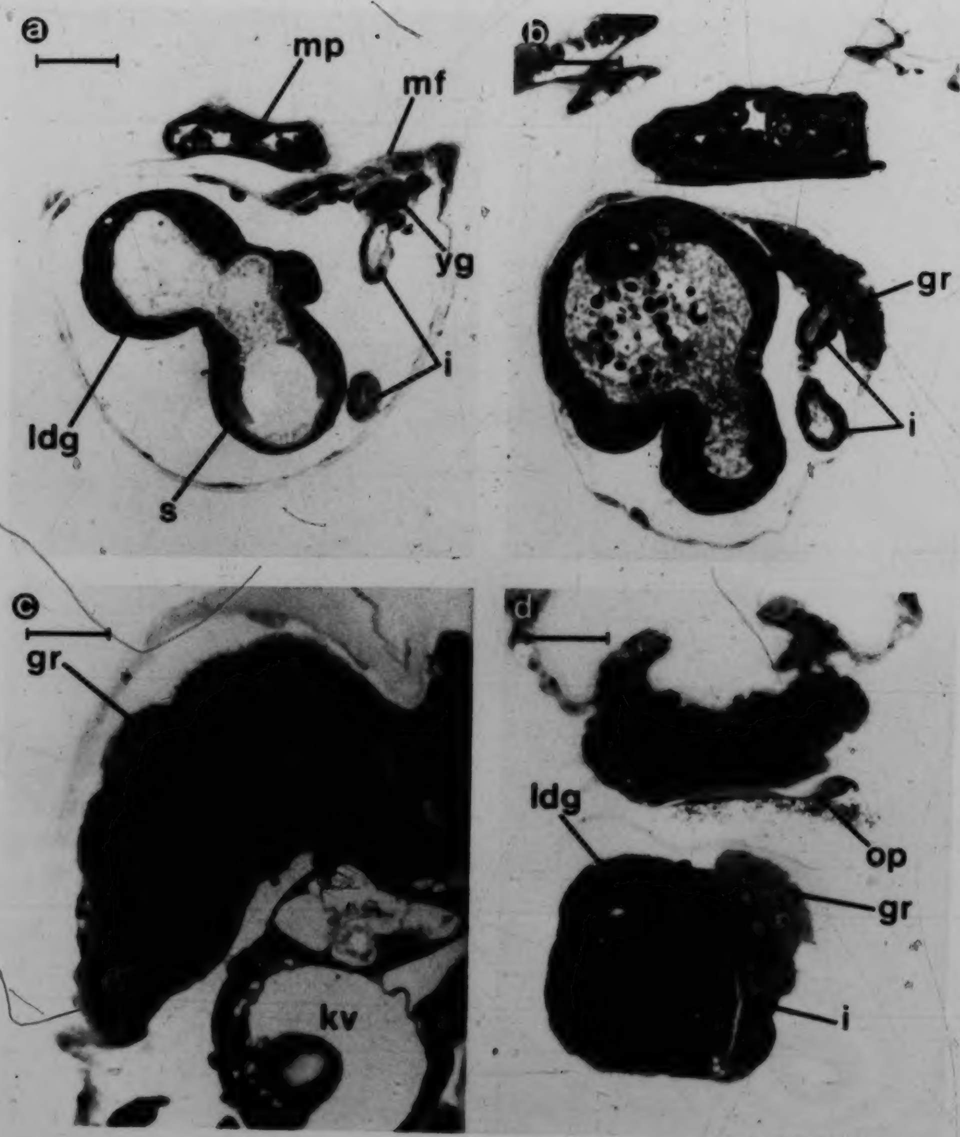

a
mp
mf
yg
i
ldg
s
b
gr
i
c
gr
kv
d
ldg
op
gr
i

Figure 14: Developmental changes in the mantle fold of the veliger

a. Frontal section of a stage II veliger showing the condition of the mantle fold epithelium at the time of its retraction from the aperture of the larval shell. The depth of the mantle cavity is considerably increased at the time of mantle retraction. Scale bar = 20 µm

b. High magnification of the mantle fold at stage II. Both the epithelial layers are composed of squamous cells. Scale bar = 10 µm

c. Sagittal section of a stage III veliger. Scale bar = 20 µm

d. High magnification of the mantle fold at stage III. The cells of the inner layer of epithelium have become cuboidal in shape. Scale bar = 10 µm

e. Frontal section of a competent veliger (stage IV) showing the hypertrophied mantle fold on the right side of the veliger. The mantle fold is not reflected, but encompasses a deep mantle cavity. Scale bar = 20 µm

f. High magnification of the mantle fold at stage IV. Note that the outer layer of the fold is composed of squamous cells while the inner layer is composed of columnar cells. Unicellular gland cells have differentiated within the hypertrophied epithelial layer, and a ciliated tract is located at the apex of the fold. Scale bar = 10 µm

Legend:

an - anus
cit - ciliated tract
e - esophagus
gr - gonadal rudiment
i - intestine
ldg - left digestive gland
lr - larval retractor muscle
mf - mantle fold
mfc - cuboidal/columnar layer of mantle fold
mfg - unicellular gland cells of mantle fold
mfs - squamous layer of mantle fold
pvm - perivisceral membrane
sd - dorsal stomach

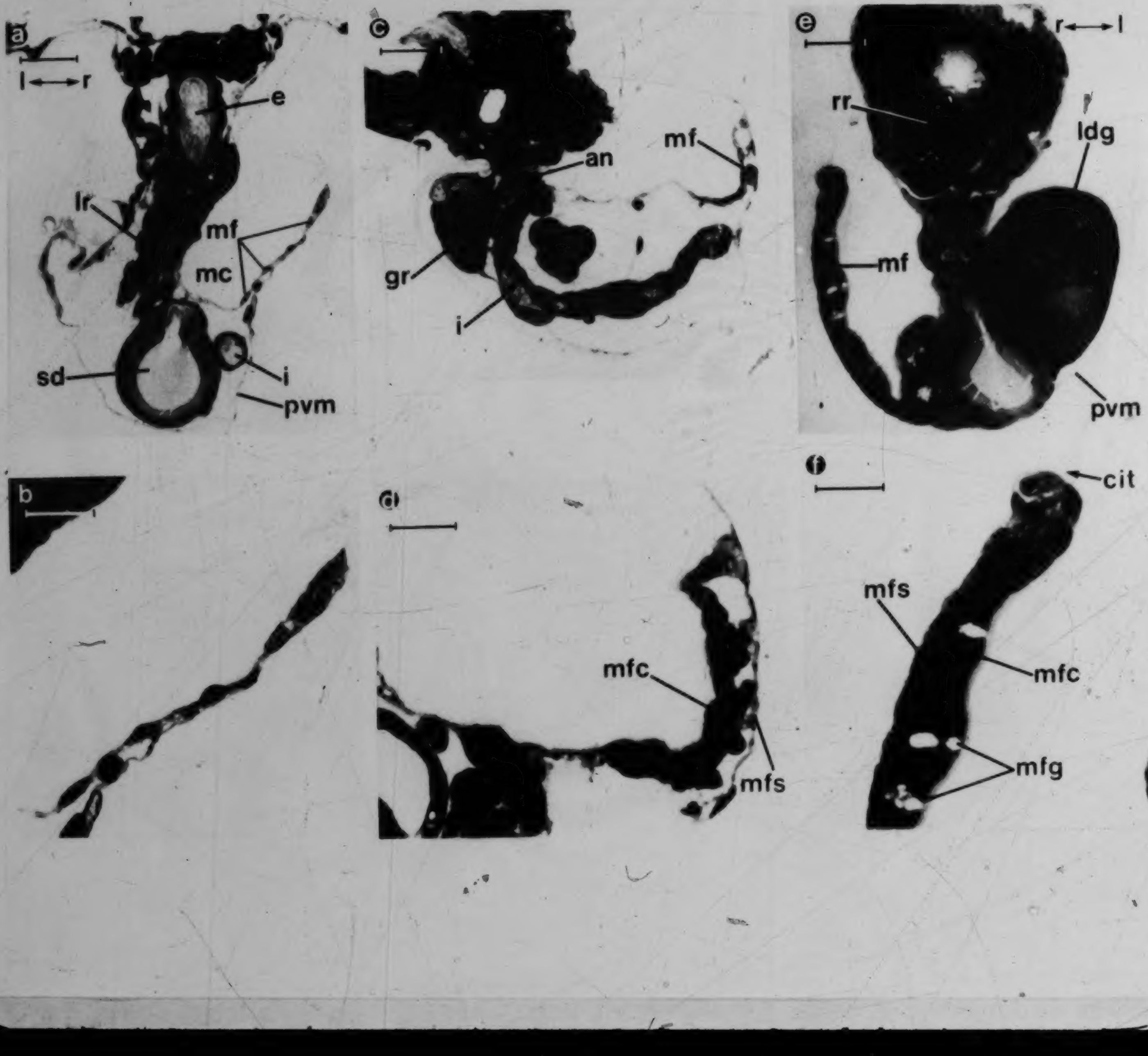

a
l ↔ r
e
lr
mf
mc
sd
i
pvm
b
c
an
mf
gr
i
d
mfc
mfs
e
r ↔ l
rr
ldg
mf
pvm
f
cit
mfs
mfc
mfg

Figure 15: Mantle retractor muscles

a. Sagittal section of a newly hatched veliger (stage I) showing a mantle retractor muscle fiber inserting on the mantle fold epithelium and on the perivisceral membrane. Scale bar = 8 μm

b. Sagittal section of a newly hatched veliger (stage I) showing a mantle retractor muscle with insertion sites on the mantle fold, intestine, and stomach. Scale bar = 15 μm

c. Frontal section of a newly hatched veliger (stage I) showing a mantle retractor muscle inserting on components of the kidney complex and on the perivisceral membrane. Scale bar = 8 μm

Legend:

i - intestine
kv - larval kidney vesicle
mf - mantle fold
mr - mantle retractor muscle
pvm - perivisceral membrane
s - stomach
st - statocyst

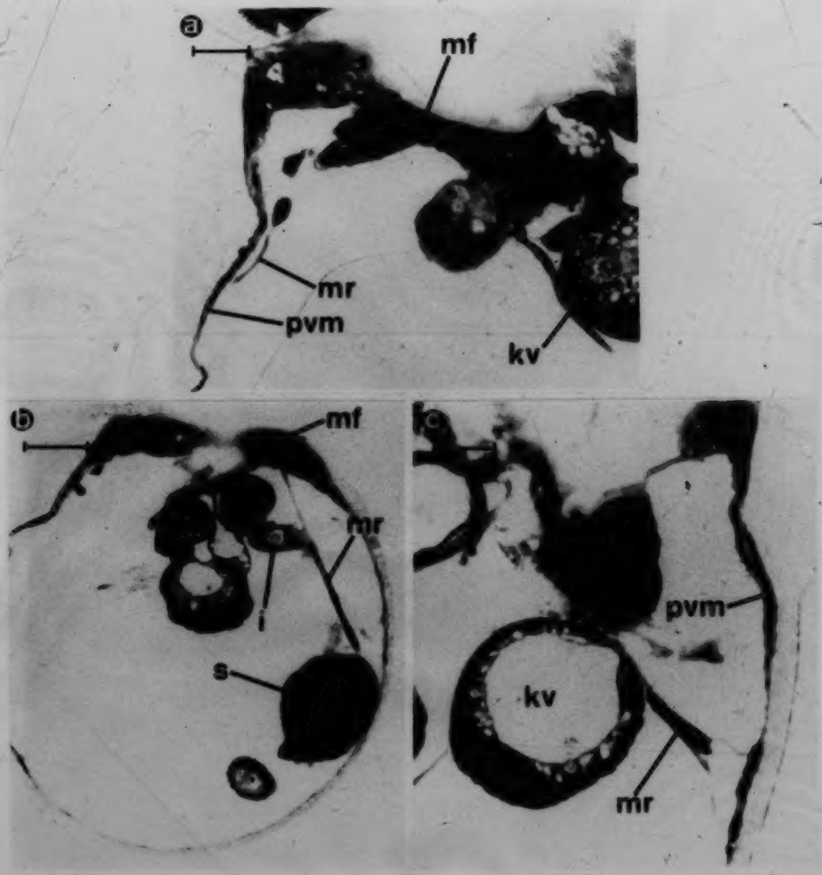

a
mf
mr
pvm
kv
b
mf
mr
i
s
c
pvm
kv
mr

Figure 16: Larval Retractor Muscle

a. Sagittal section of a newly hatched larva (stage I) showing the larval retractor muscle. The muscle originates on the posterior wall of the shell (and perivisceral membrane) and divides into 3 main branches. The pedal branch and one of the two velar branches can be seen in this section. Note the insertion of the pedal branch onto the tissues of the foot and the perivisceral membrane at the ventral aperture of the larval shell. Scale bar = 20 μm

b. Frontal section of a stage II veliger showing the hypertrophy of the trunk of the larval retractor (compared to the newly hatched condition) and the two velar branches of the muscle extending into the velar lobes. Scale bar = 20 μm

c. Cross section of a stage III veliger. The main body of the larval retractor muscle (lm) runs on the left, dorso-lateral side of the esophagus. The section transects the pedal branch (arrows) as it moves ventral to the esophagus to insert on the pedal tissues and on the perivisceral membrane at the ventral aperture of the shell. Scale bar = 15 μm

d. Cross section of a competent larva (stage IV) passing through a region close to the origin of the larval retractor muscle. Note the intimate association between the larval retractor muscle and the hypertrophied mantle fold, the dorsal stomach, and the posterior extremity of the left digestive gland. Scale bar = 8 μm

e. Sagittal section of a competent veliger (stage IV) showing the marked enlargement, relative to stage I, of the larval retractor muscle. Note the relative, premetamorphic positions of the retractor muscle, the mantle fold, and the left digestive gland. Scale bar = 20 μm

Legend:

e - esophagus
gr - gonadal rudiment
i - intestine
ldg - left digestive gland
lr - larval retractor muscle
mf - mantle fold
mp - metapodium
op - operculum
plr - pedal branch
pvm - perivisceral membrane
rr - radular rudiment
sd - dorsal stomach
v - velum
vlr - velar branch

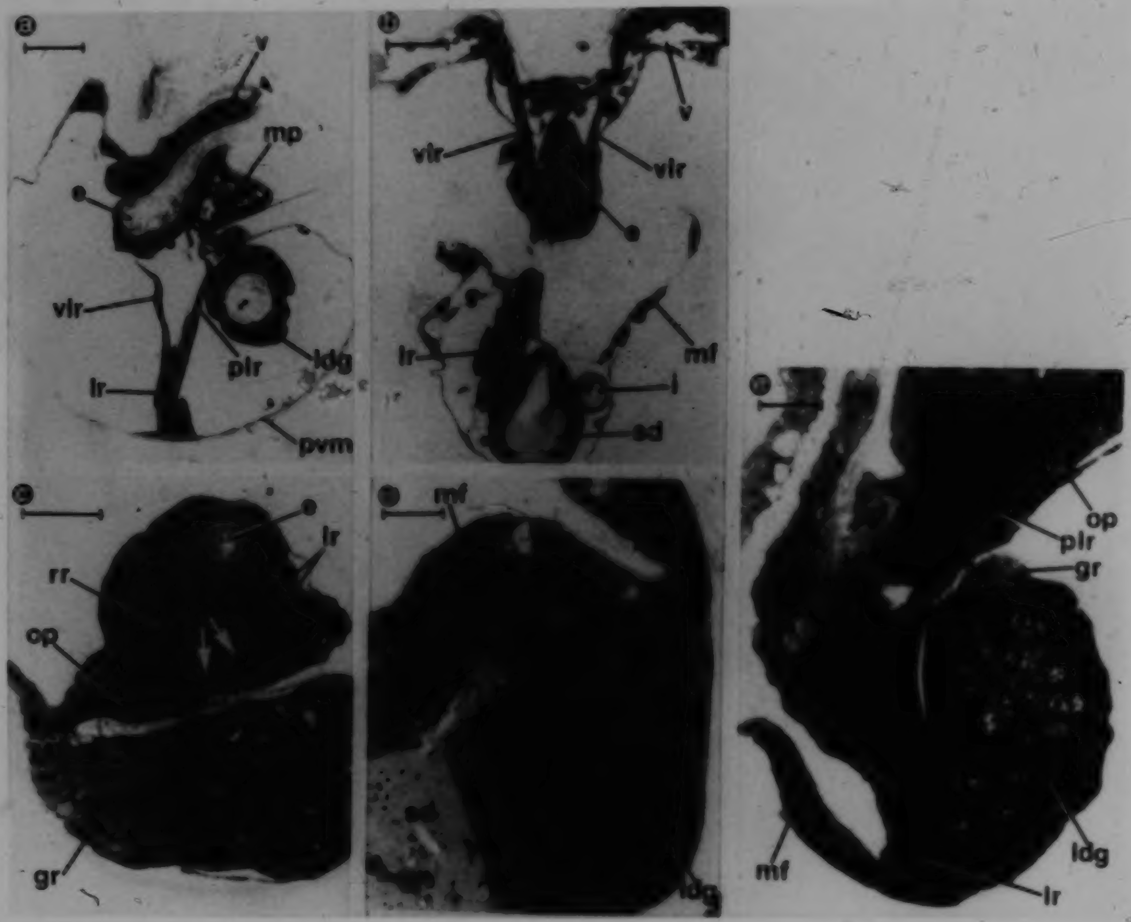

a
v
mp
e
vlr
lr
plr
ldg
pvm
b
v
vlr
vlr
e
lr
mf
l
sd
c
e
lr
rr
op
gr
d
mf
ldg
e
op
plr
gr
ldg
mf
lr

Figure 17: Size class profiles for three, temporally separated populations of D. steinbergae. Shaded regions indicate individuals with a notal length of less than 0.5 mm.

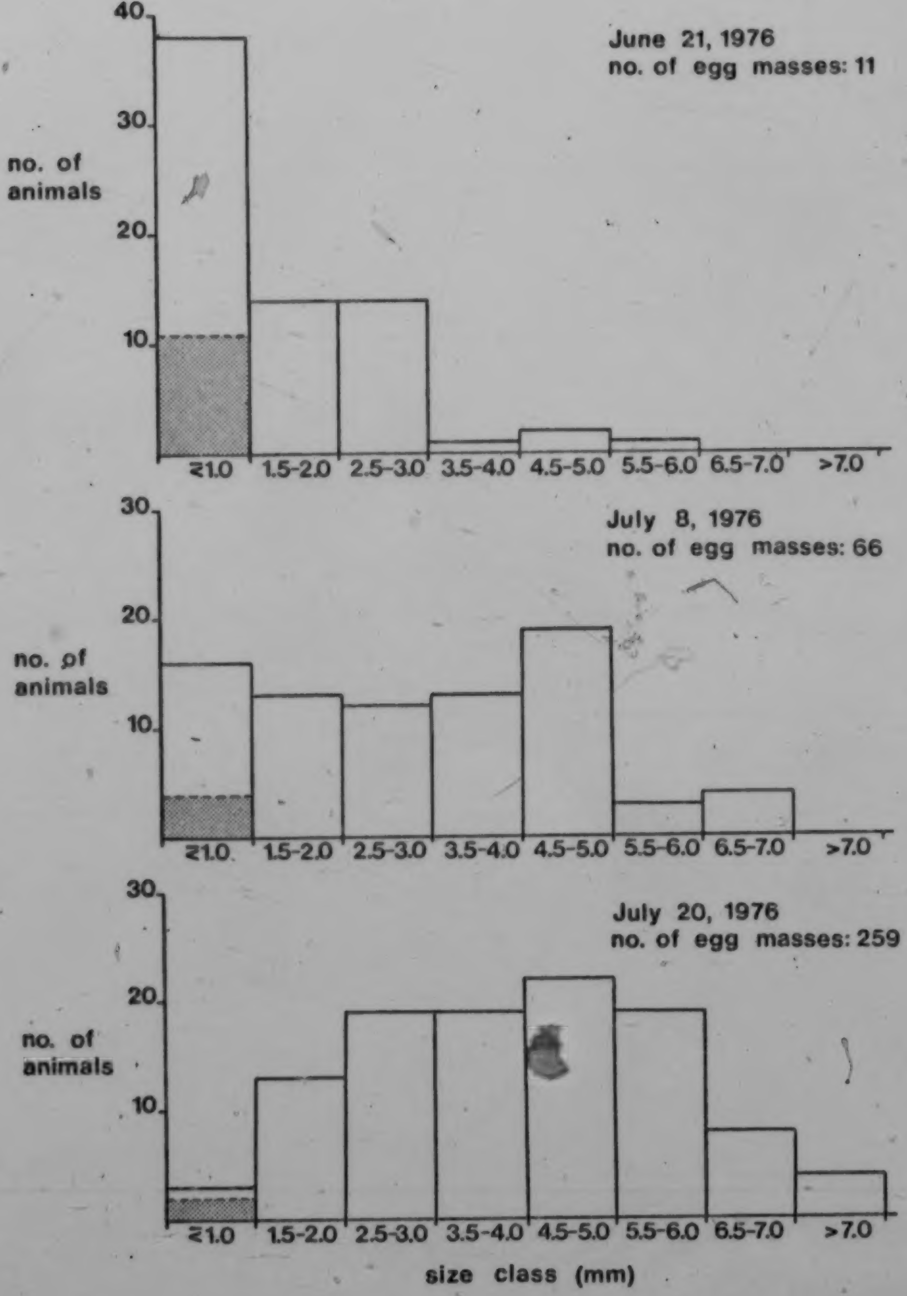

June 21, 1976
no. of egg masses: 11
no. of animals
40
30
20
10
≥1.0
1.5-2.0
2.5-3.0
3.5-4.0
4.5-5.0
5.5-6.0
6.5-7.0
>7.0
July 8, 1976
no. of egg masses: 66
no. of animals
30
20
10
≥1.0
1.5-2.0
2.5-3.0
3.5-4.0
4.5-5.0
5.5-6.0
6.5-7.0
>7.0
July 20, 1976
no. of egg masses: 259
no. of animals
30
20
10
≥1.0
1.5-2.0
2.5-3.0
3.5-4.0
4.5-5.0
5.5-6.0
6.5-7.0
>7.0
size class (mm)

Figure 18: Effect of delayed metamorphosis on larval response to the inducing effect of *M. villosa*. Broken lines indicate periods during which hourly observations were not made.

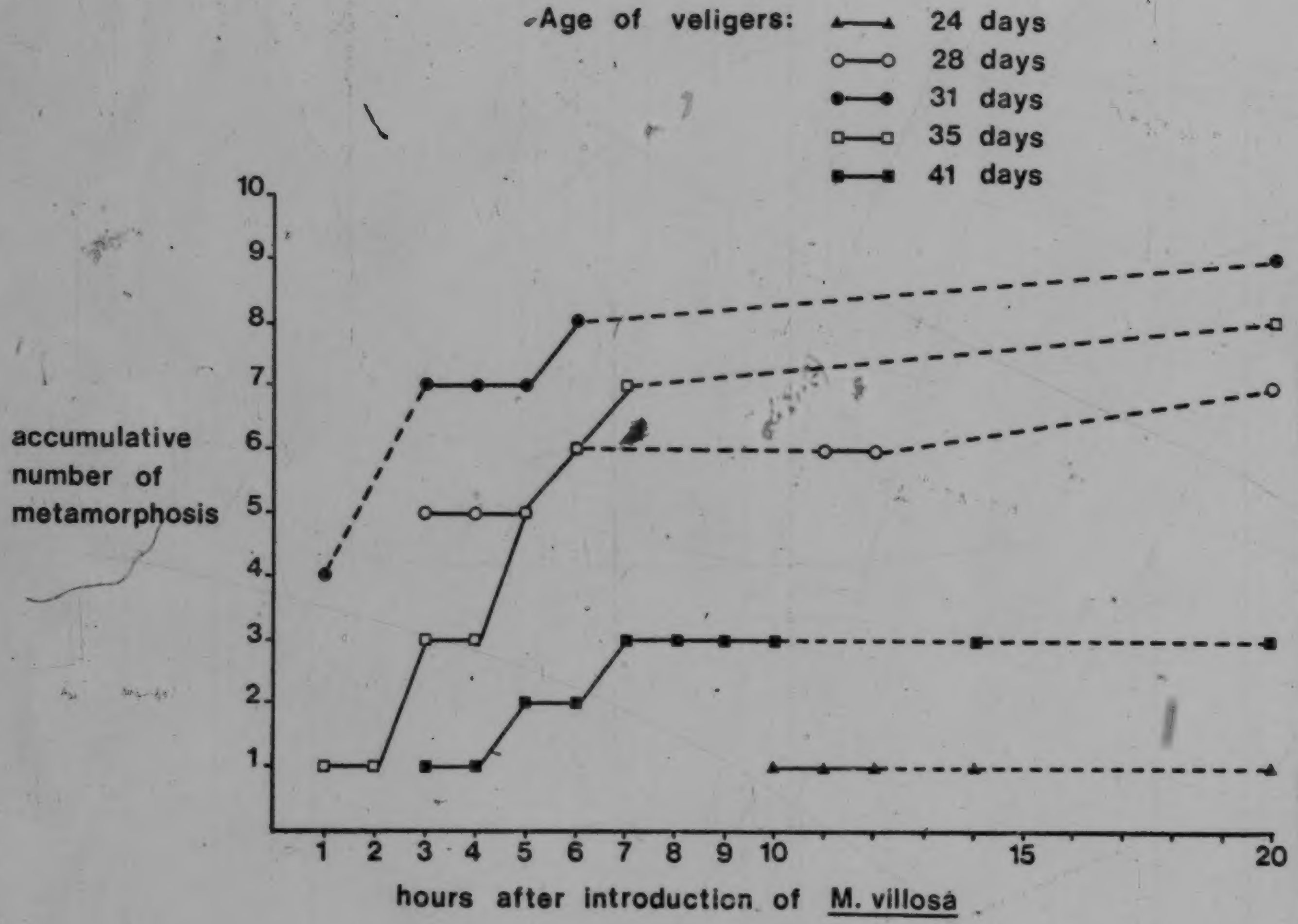
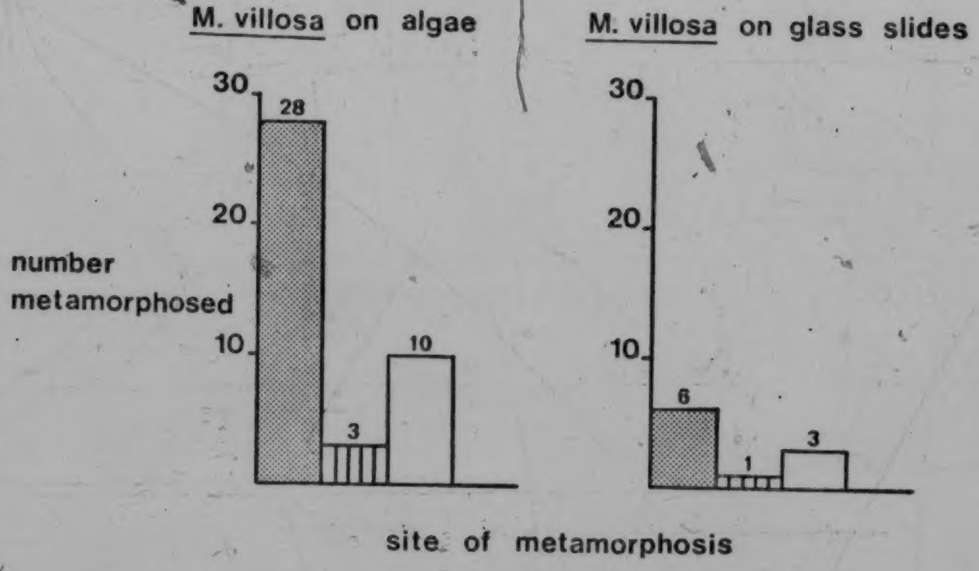

Age of veligers:
24 days
28 days
31 days
35 days
41 days
accumulative number of metamorphosis
1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
6
7
8
9
10
15
20
hours after introduction of M. villosa

Figure 19: Preference for the periphery of *M. villosa* colonies as the site of metamorphosis. The sites at which metamorphosis occurred are grouped into three categories as follows:

▒ on algae with anterior end apposed to the colony periphery of *M. villosa*.

▥ on undifferentiated zoids

□ on differentiated zoids


M. villosa on algae
M. villosa on glass slides
30
20
10
28
3
10
30
20
10
6
1
3
number metamorphosed
site of metamorphosis

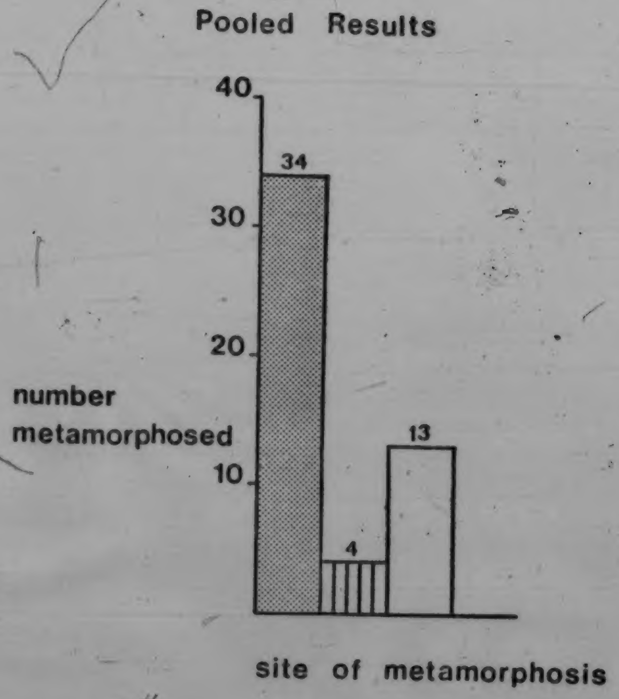

Pooled Results
40
30
20
10
34
4
13
number metamorphosed
site of metamorphosis

Figure 20: Maps of four induction substrates. The bryozoan colonies, which are represented by the stippled areas, are encrusted on L. saccharina. The asterisks represent metamorphic sites; those which occurred in indentations along the periphery of the colonies are indicated by arrows.

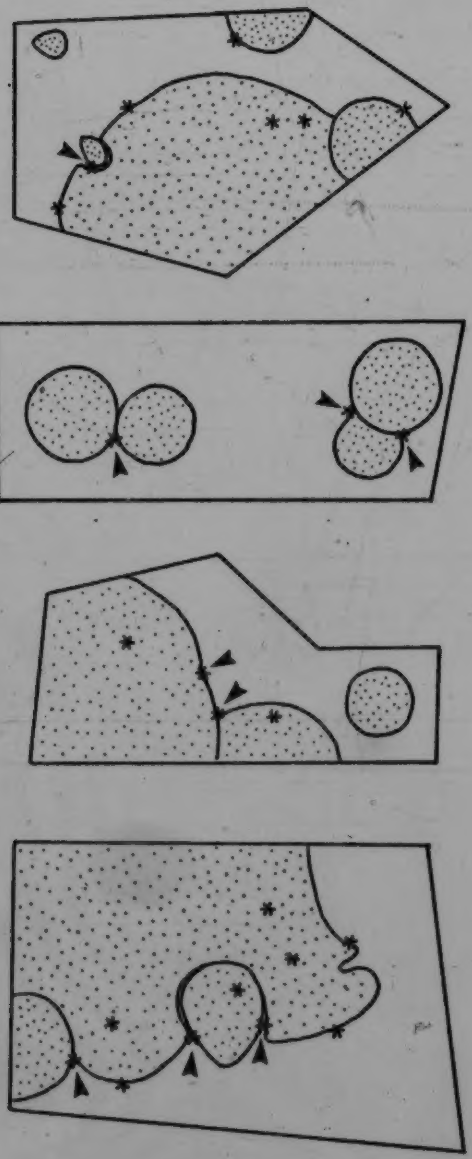


Figure 21: Metamorphosis of a *D. steinbergae* veliger; initial events. The time interval from a to f is 42 minutes.

a. Competent veliger positioned with its foot on the surface of *Laminaria*, and its anterior end apposed to the periphery of a colony of *Membranipora*.
Scale bar = 100 µm

b. Onset of metamorphosis. Note the detachment of the visceral mass from the inner wall of the shell whorl and the loss of the operculum.

c. Loss of the larval kidney vesicle and the velar cells

d - f. Withdrawal of the visceral mass from the larval shell.

Legend:

L - *Laminaria saccharina*
M - *Membranipora villosa*
kv - larval kidney vesicle
op - operculum
s - stomach
sh - shell
v - velum
vm - visceral mass

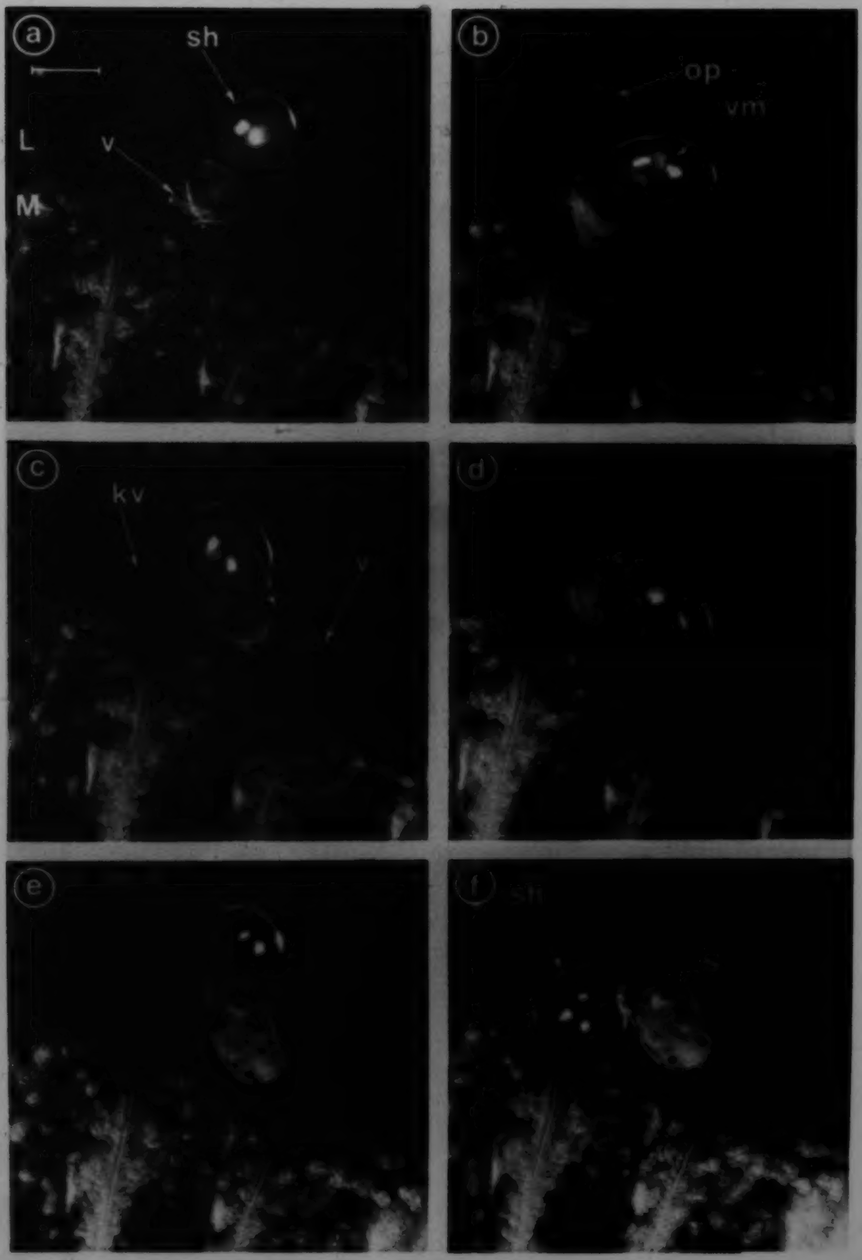

a
sh
L
v
M
b
op
vm
c
kv
v
d
e
f
sh

Figure 22: Metamorphosis: Changes in body shape and repositioning of the larval retractor muscle, stomach, and digestive gland.

a. Crawling, stage IV veliger; right lateral view (the larval kidney cells are omitted). Note that the stomach lies ventral to the anterio-posterior axis of the larva.

b. Crawling, stage IV veliger;left lateral view.

c. Illustration of shell pivoting behavior which occurs just prior to shell loss. The sole of the foot remains in contact with the substrate along its entire length.

d. Immediately after shell loss. Note anterior displacement of the larval retractor muscle and the anterior rotation of the stomach and the digestive gland. The stomach and part of the digestive gland are located dorsal to the anterio-posterior axis of the post-larva at this stage.

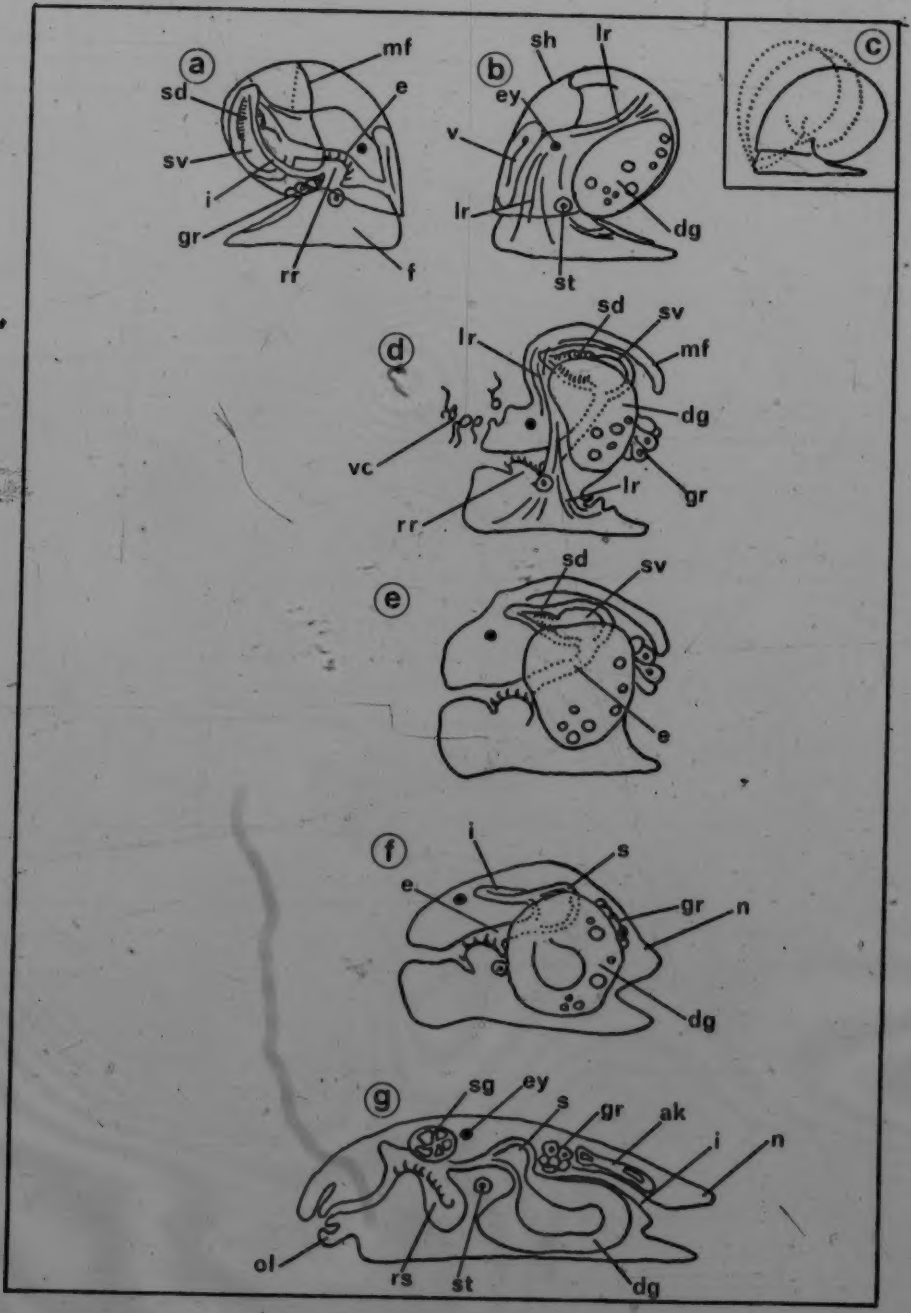
e. Thirty minutes after shell loss.

f. Five hours after shell loss. Note the progressive incorporation of the digestive gland into the foot, resulting in a broadening of the connection between the visceral hump and the foot.

g. Three day old juvenile. The body has become dorsoventrally flattened.

Legend:

ak - adult kidney
dg - digestive gland
e - esophagus
ey - eye
f - foot
gr - gonadal rudiment
i - intestine
lr - larval retractor muscle
mf - mantle fold
n - notum
ol - oral lips
rr - radular rudiment
rs - radular sac
sd - dorsal stomach
sh - larval shell
sg - salivary gland
st - statocyst
sv - ventral stomach
v - velum
vc - velar cells


a
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mf
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ak
i
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ol
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dg

Figure 23: Metamorphosis: detorsion and formation of the notum

a. Sagittal section of a post-larva immediately after shell loss; section passes to the left of the midline. The trunk of the larval retractor muscle has been pulled anteriorly and the mantle fold has reflected posteriorly. As a result, the columnar layer of the mantle fold (which contains the unicellular gland cells) faces externally. Note the velar cells dissociating from the surrounding cephalic epithelium. Scale bar = 20 μm

b. Cross section of a post-larva immediately after shell loss. The stomach is located on the dorso-lateral face of the digestive gland. The mantle tissue is attached to the body of the larva on the right side but is free on the left side. The anus is not yet located in a terminal position, and the type b larval kidney cell is still present. Scale bar = 20 μm

c. Sagittal section of a post-larva at 30 minutes after shell loss. The intestine opens in a terminal position, below the free edge of the mantle fold. The type d larval kidney cells are still distinguishable. Note that the digestive gland has become incorporated into the foot, resulting in a broadening of the connection between the visceral hump and the foot. This process is possible due to previous loss of the shell and operculum. The ducts of the pedal glands are filled with secretory product. Scale bar = 20 μm

d. Sagittal section of a post-larva at 5 hours after shell loss. The free edge of the reflected mantle fold has fused with the dorsal wall of the anal aperture, thus placing the anus below the tissue of the newly formed notum. Scale bar = 20 μm

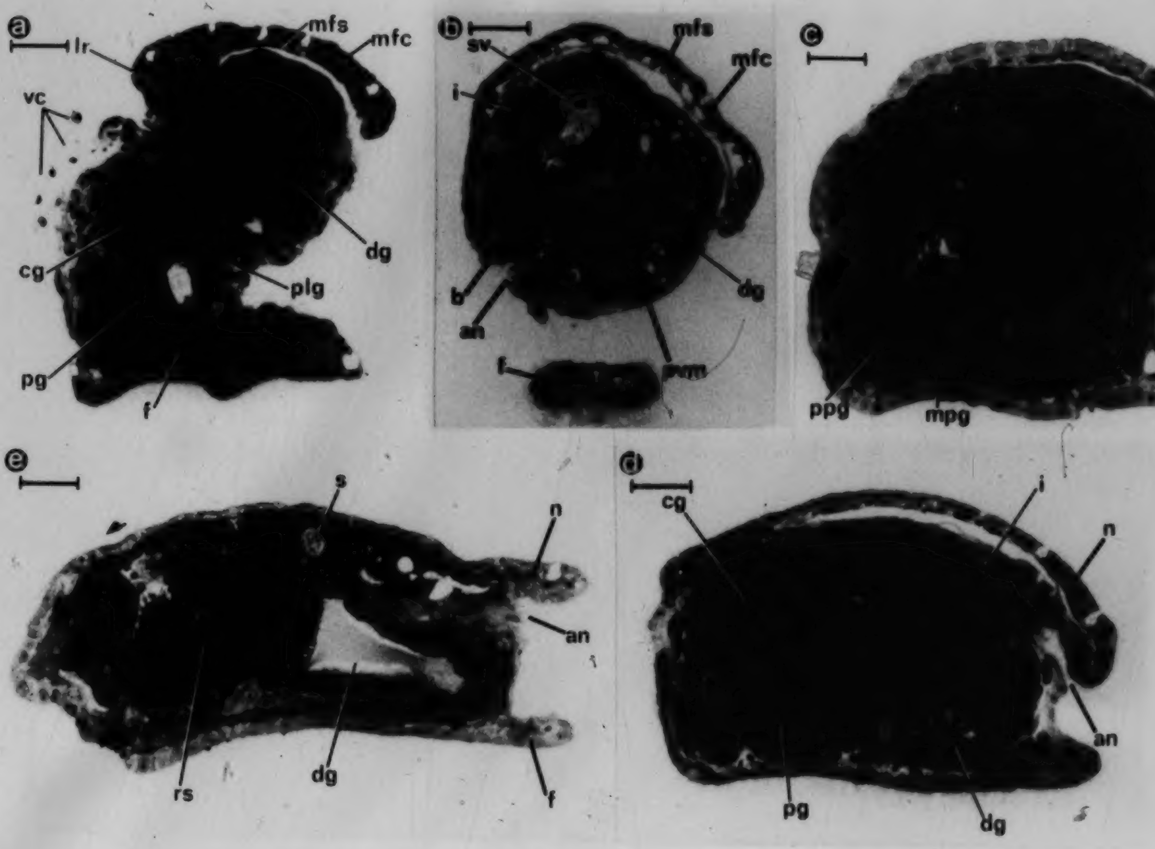
e. Sagittal section of a 48 hour old post-larva. The intestine and the kidney extend posteriorly over the mid-dorsal surface of the digestive gland. Proliferation of the notal cells (formerly the mantle fold) has produced a skirt of notal tissue which overlaps the anus and foot. Scale bar = 20 μm

Legend: continued on following page

Figure 23: continued

Legend:

an - anus
b - type b larval kidney cell
cg - cerebral ganglion
d - type d larval kidney cell
dg - digestive gland
f - foot
i - intestine
lr - larval retractor muscle
mf - mantle fold
mfc - columnar layer of mantle fold
mfs - squamous layer of mantle fold
mpg - metapodial gland
n - notum
pg - pedal ganglion
plg - pleural ganglion
ppg - propodial gland
pvm - perivisceral membrane
rs - radular sac
s - stomach
sv - ventral stomach
vc - velar cells


a
lr
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vc
dg
cg
plg
pg
f
b
sv
mfs
mfc
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b
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dg
f
pvm
c
mf
i
d
ppg
mpg
e
s
n
an
rs
dg
f
d
cg
i
n
an
pg
dg

Figure 24: Metamorphosis: Detorsion and the subnotal positioning of the anus

a. A crawling stage IV veliger illustrating the various components of the mantle and perivisceral epithelium which are associated with the anus and the trunk of the larval retractor muscle (see Legend).

b. Immediately after shell loss; lateral view. The larval kidney vesicle has been shed, the stomach and the trunk of the larval retractor muscle have pivoted anteriorly (compare position of the star (☆) in figures a and b), and the mantle fold has reflected.

c. Immediately after shell loss; posterior view. Note that the free edge of the mantle fold begins at the ANTERIOR edge of the anus and then proceeds around the left side of the post-larva.

d. Five hours after shell loss; lateral view. The free edge of the mantle fold has fused with the dorsal wall of the anal aperture.

e. Five hours after shell loss; posterior view. Note that the edge of the notum on the right side is formed by the floor of the mantle cavity, while the left side is formed by the free edge of the mantle fold.

Legend:

━━━━ free edge of mantle fold (fmf)
----- mantle fold (mf)
••••••• floor of mantle cavity (fmc)
.... perivisceral membrane (pvm)
ak - adult kidney
an - anus
dg - digestive gland
f - foot
i - intestine
kv - larval kidney vesicle
lr - larval retractor muscle
n - notum
s - stomach
sh - larval shell

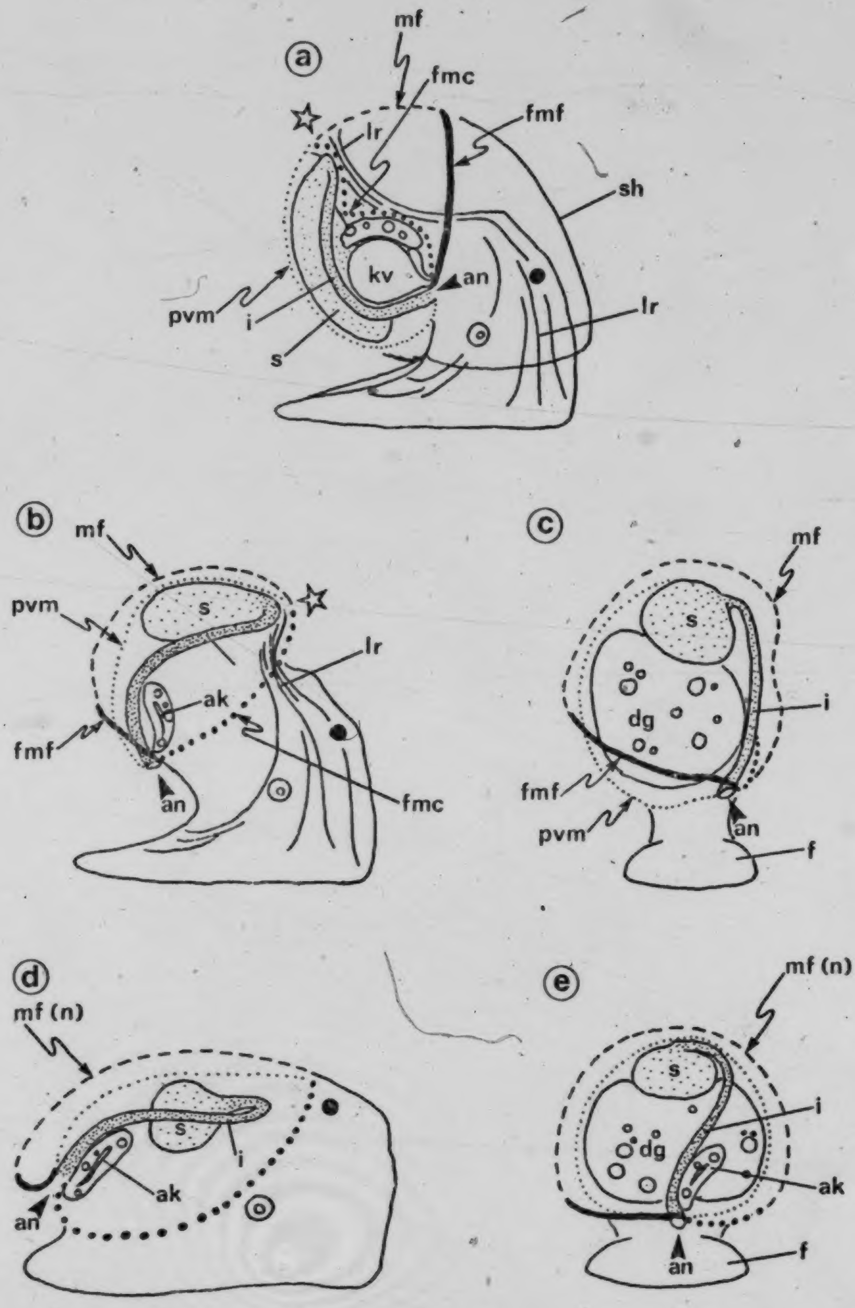

a
mf
fmc
fmf
lr
sh
kv
an
pvm
i
s
lr
b
mf
pvm
s
lr
ak
fmf
an
fmc
c
mf
s
i
dg
fmf
pvm
an
f
d
mf (n)
s
i
ak
an
e
mf (n)
s
i
dg
ak
an
f

Figure 25: Dissociation of the dorsal stomach cells during metamorphosis

a. Cross section of a post-larva immediately after shell loss. The dorsal stomach cells are identifiable by the dense border of basal bodies at their apical ends (small arrows). The large arrow indicates a dorsal stomach cell in the process of detaching from the stomach wall. The lumen of the stomach contains algal cells and dissociated dorsal stomach cells. Scale bar = 5 µm

b. Cross section of a post-larva immediately after shell loss showing algal and dissociated dorsal stomach cells in the lumina of the stomach and digestive gland (arrows). Scale bar = 20 µm

c. Sagittal section of a post-larva 30 minutes after shell loss. The dissociating dorsal stomach cells are indicated by arrows. Note that the dissociation process is occurring at the junction between the ventral and dorsal stomachs. Scale bar = 5 µm

Legend:

ac - algal cell
dg - digestive gland
e - esophagus
f - foot
i - intestine
sd - dorsal stomach
sdc - dorsal stomach cells (dissociated)
sv - ventral stomach

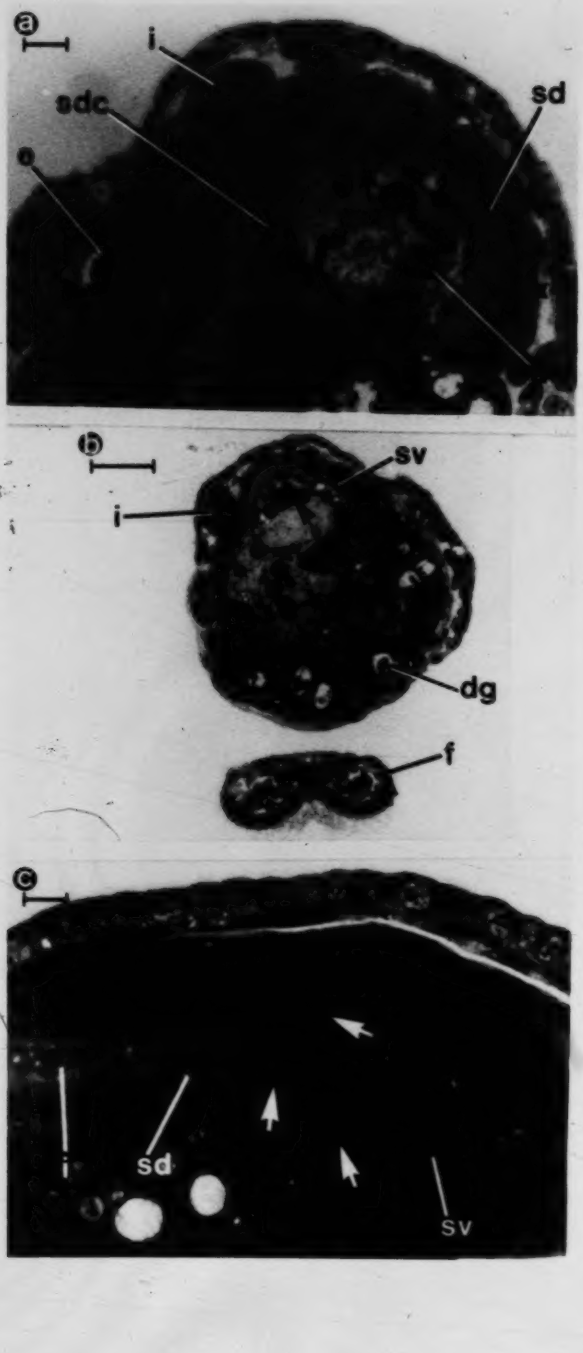

a
i
sdc
sd
e
b
sv
i
dg
f
c
i
sd
sv

Figure 26: Later metamorphic changes in the stomach

a. Sagittal section of a post-larva 5 hours after shell loss. Note that the intestine joins directly to the ventral stomach due to the previous loss of the dorsal stomach. Scale bar = 20 μm

b. Sagittal section of a post-larva 48 hours after shell loss. Note absence of the ventral stomach. Scale bar = 20 μm

c. Cross section of a 10 day old juvenile. The stomach is a simple sac, internally ciliated, and located on the mid-dorsal surface of the digestive gland. The section passes through the anterior region of the stomach where it is connected to both the esophagus and the intestine. Scale bar = 75 μm

Legend:

dg - digestive gland
e - esophagus
i - intestine
mpg - metapodial glands
n - notum
olg - oral lip gland
peg - pedal glands
ppg - propodial gland
pv - phagocytic vacuole
s - stomach
sv - ventral stomach

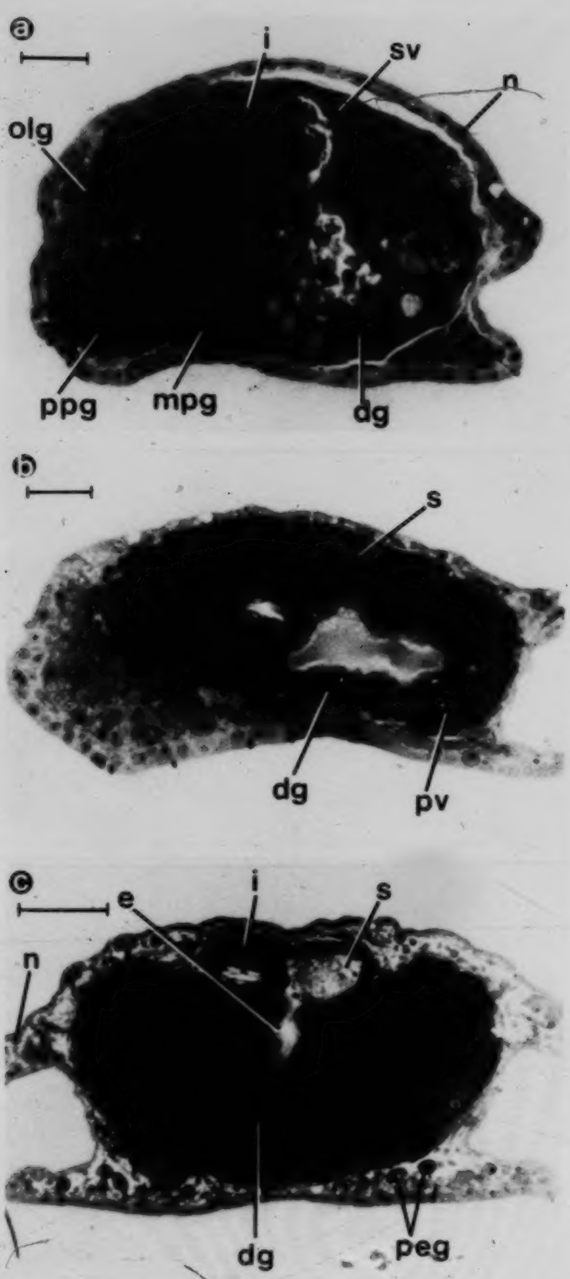

a
i
sv
n
olg
ppg
mpg
dg
b
s
dg
pv
c
e
i
s
n
dg
peg

Figure 27: Development of the buccal pump

a. Cross section through the anterior portion of a post-larva 5 hours after shell loss. Note the hypertrophied cells of the prospective buccal pump, and the laterally compressed lumen of the esophagus. Scale bar = 5 µm

b. Cross section through the developing buccal pump of a 48 hour old post-larva. Note the slit-like lumen of the buccal pump and the cuticle which lines its lumen. Scale bar = 20 µm

c. Cross section through the buccal pump and anterior portion of the radula in a 10 day old juvenile. Note the extensive development of the muscles associated with the buccal mass. Scale bar = 20 µm

Legend:

bp - buccal pump
cc - cerebral commissure
cg - cerebral ganglion
cu - cuticle
ey - eyespot
om - odontophore muscles
pbp - prospective buccal pump
pg - pedal ganglion
rt - radular teeth

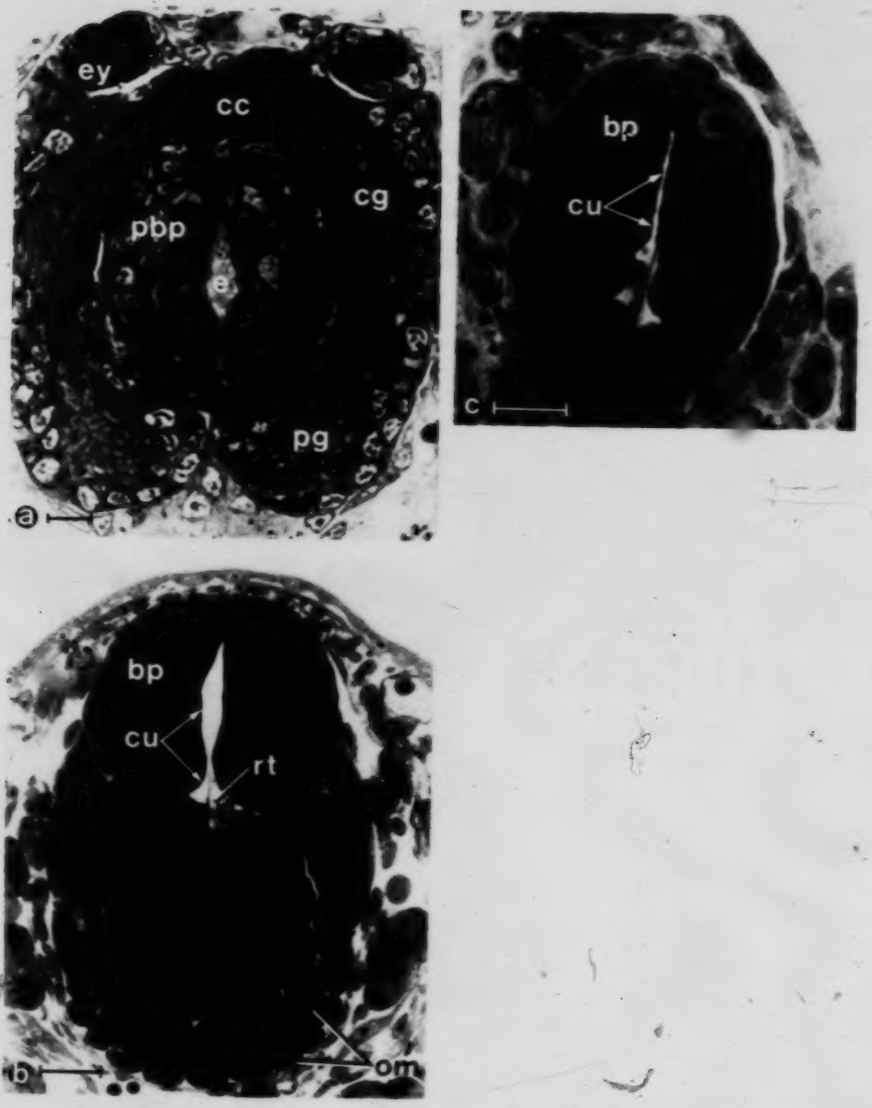

ey
cc
cg
pbp
e
pg
a
bp
cu
c
bp
cu
rt
b
om

Figure 28: Development of the oral lip glands

a. Sagittal section through the anterior end of a post-larva at 1/2 hour after shell loss. Note the rudimentary oral lip glands which lie on the dorso-lateral sides of the esophagus. Scale bar = 8 µm.

b. Cross section through the oral region of a 10 day old juvenile, showing the oral lips surrounding the mouth and the enlarged oral lip glands. Scale bar = 25 µm

Legend:

dol - ducts of the oral lip glands
mo - mouth
n - notum
ol - oral lips
olg - oral lip glands
ot - oral tentacles
ppg - propodial gland

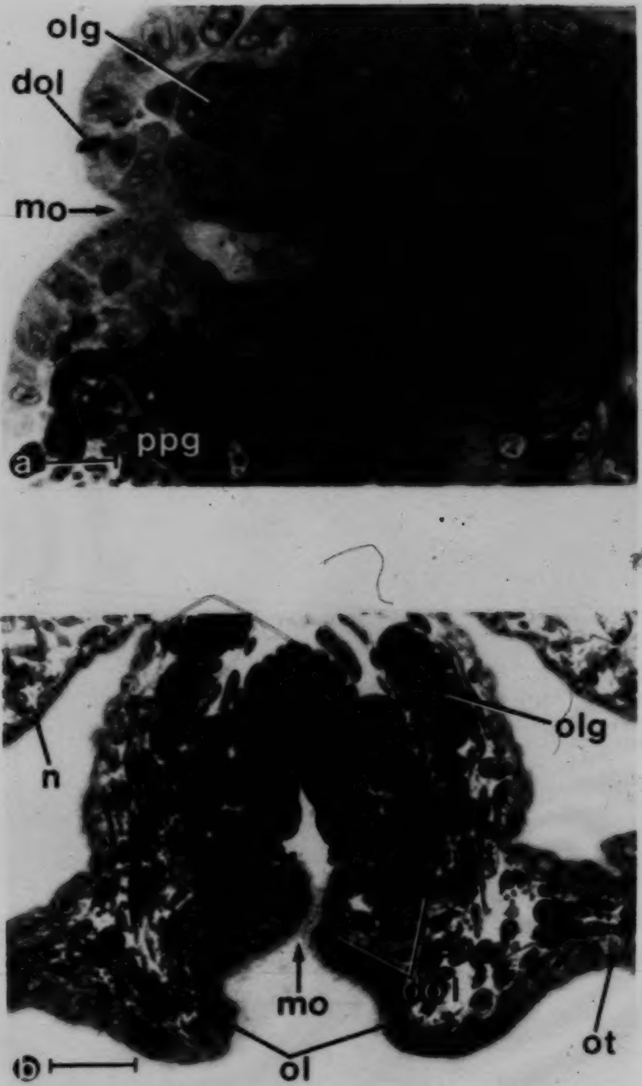

olg
dol
mo
ppg
a
olg
n
mo
dol
ot
ol
b

Figure 29: Development of the salivary glands

a. Cross section of a 48 hour old post-larva through the region just posterior to the buccal pump. The salivary glands are evident as small outpocketings of the lateral walls of the esophagus. Some of the cells of the evaginations contain secretory material.
Scale bar = 5 µm

b. Sagitall section of a 3 day old juvenile. The salivary glands have enlarged considerably. Scale bar = 5 µm

c. Cross section through the buccal mass of a 10 day old juvenile (just posterior to the buccal pump). The large salivary glands encircle the esophagus and radular sac. Scale bar = 50 µm

Legend:

e - esophagus
f - foot
mo - mouth
n - notum
rs - radular sac
sg - salivary gland

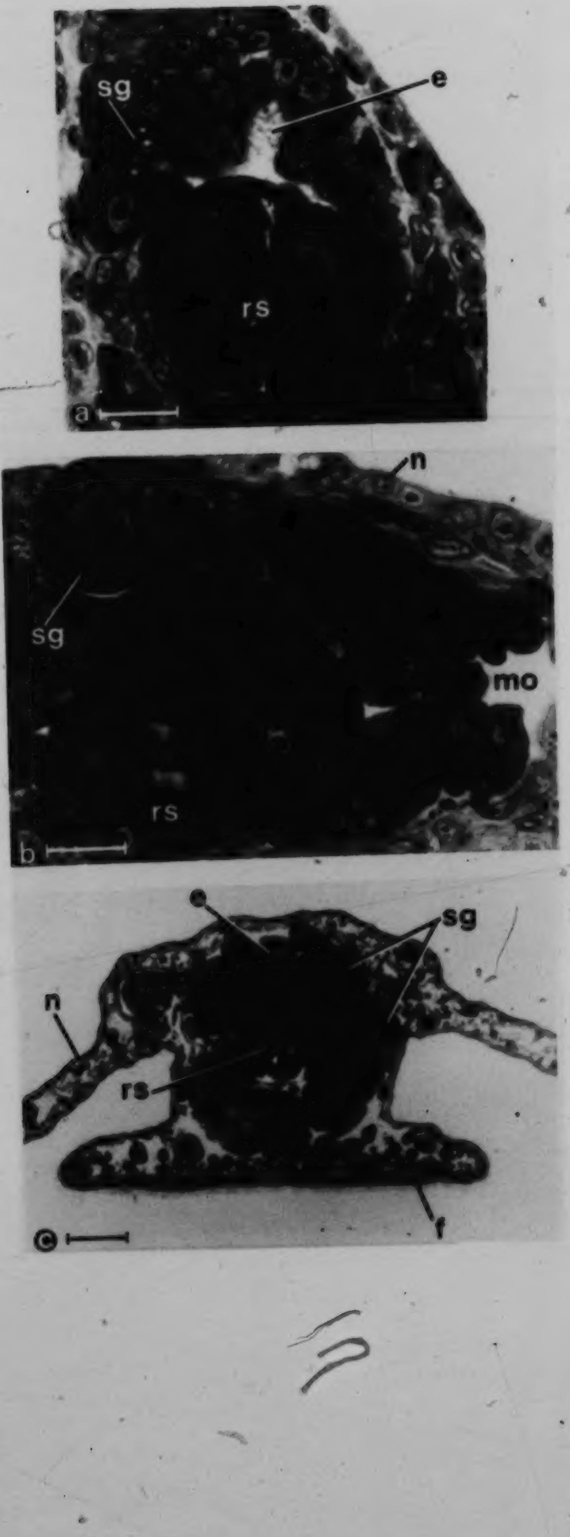

sg
e
rs
a
n
sg
mo
rs
b
e
sg
n
rs
f
c

Figure 30: Metamorphic transformation of the digestive gland

a. Digestive gland immediately after shell loss. Note the dorsal stomach cells and the algal cells in the lumen. Scale bar = 10 µm

b. Digestive gland at 5 hours after shell loss. Note the dorsal stomach cell and the algal cells within phagocytic vacuoles. Scale bar = 10 µm

c. Digestive gland at 48 hours after shell loss. Note the phagocytic vacuole and the differentiation of zymogen cells. Scale bar = 10 µm

d. Digestive gland of a 10 day old juvenile. Scale bar = 10 µm

Legend:

ac - algal cell
fv - flocculent vacuole
i - intestine
lv - lipoid vesicle
pv - phagocytic vacuole
sdc - dorsal stomach cell
zc - zymogen cell

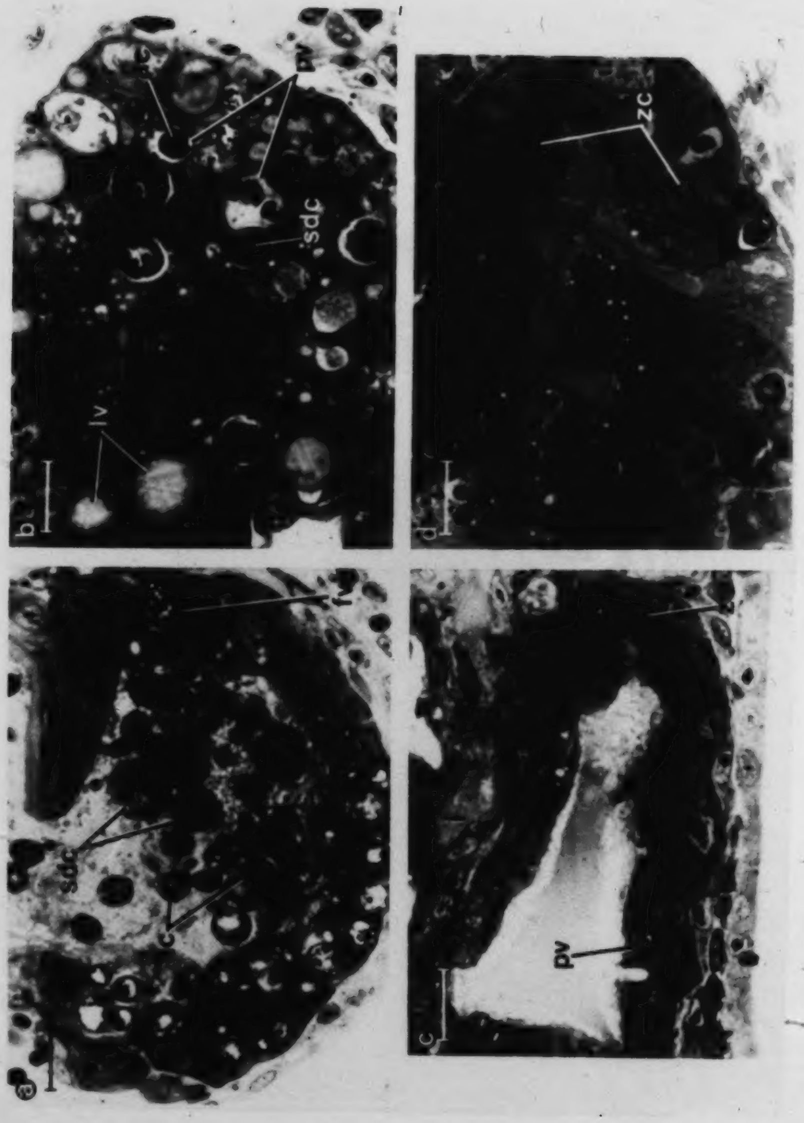

b
lv
sdc
pv
d
zc
a
sdc
c
c
pv

Figure 31: Degeneration of the larval retractor muscle during metamorphosis

a. Cross section through the anterior region of a post-larva, shortly after shell loss. Note the position of the larval retractor muscle after removal of the visceral mass from the shell. Scale bar = 20 μm

b. Enlargement of a, showing the disorientation of muscle fibers within the larval retractor muscle. Scale bar = 10 μm

c. Cross section through the anterior region of a post-larva at 30 minutes after shell loss. Note the further deterioration of the larval retractor muscle. Scale bar = 10 μm

Legend:

cg - cerebral ganglion
e - esophagus
f - foot
i - intestine
lr - larval retractor muscle
plg - pleural ganglion
pg - pedal ganglion
sv - ventral stomach

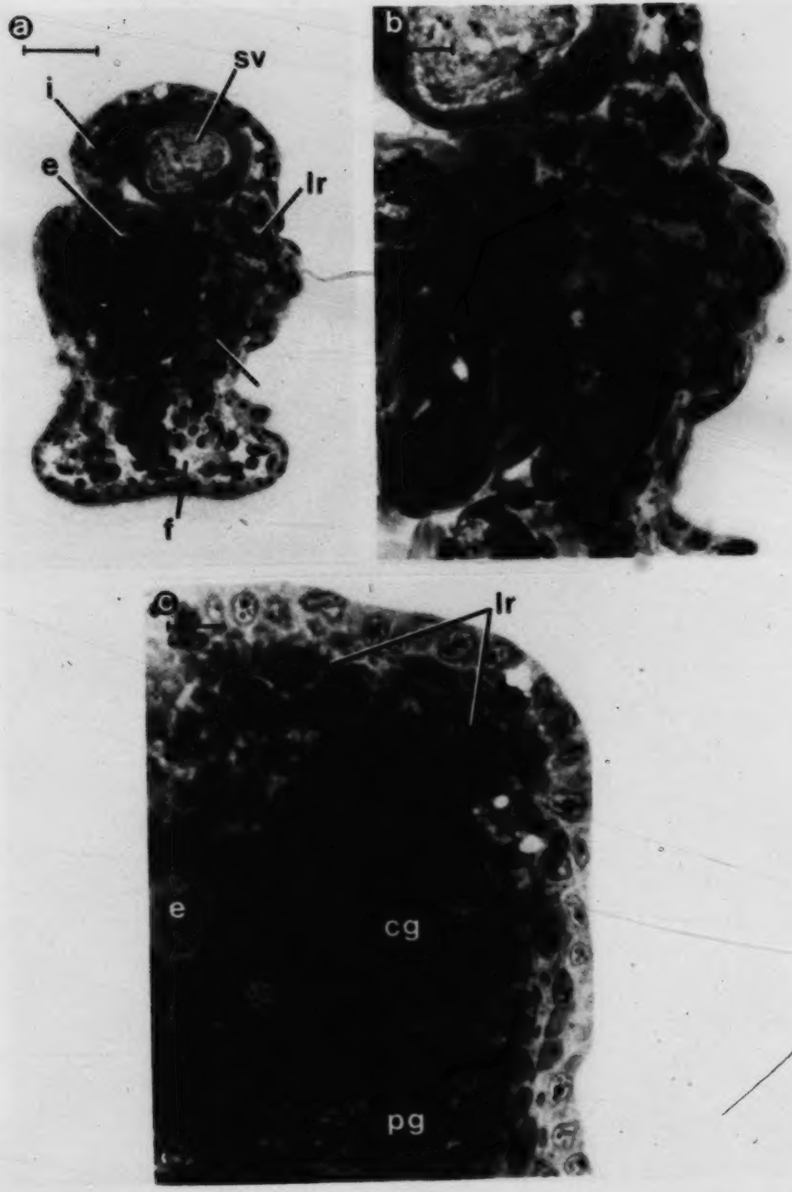

a
sv
i
e
lr
f
b
c
lr
e
cg
pg

Figure 32: Development of muscles within the juvenile

a. Cross section through the stomach of a 10 day old juvenile, showing circular muscle fibers (arrows) around the wall of the stomach. Scale bar = 8 μm

b. Cross section of a 10 day old juvenile passing through the terminal region of the body. Note the circular muscles around the rectum and the branchiae on either side of the anus. Scale bar = 20 μm

c. Cross section through the side of the body of a 10 day old juvenile. The arrows indicate the subepidermal muscle fibers of the notum and the foot, and the dorso-ventral muscles. Scale bar = 20 μm

Legend:

br - branchia
f - foot
n - notum
r - rectum
s - stomach

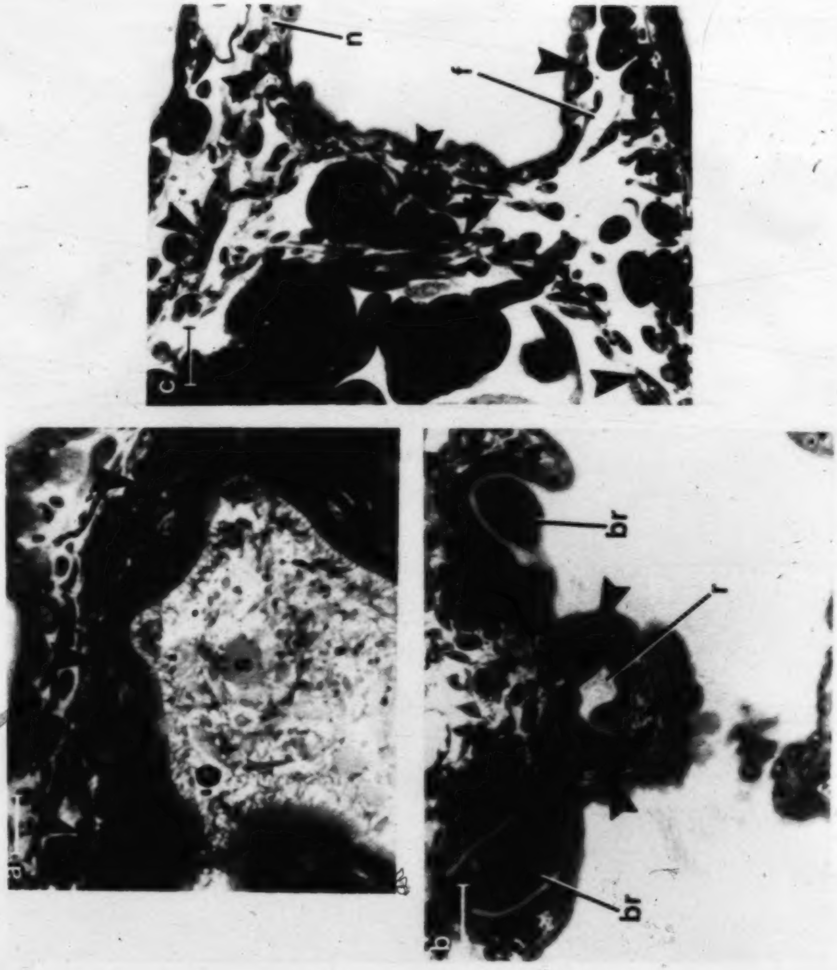

c
n
t
a
b
br
r
br

Figure 33: Development of the adult kidney

a. Sagittal section of a post-larva immediately after shell loss. The section passes to the right of the median axis. Note the posterior location of the adult kidney rudiment. The type b larval kidney cell is still present. Scale bar = 20 μm

b. Sagittal section of a post-larva at 5 hours after shell loss. The vacuolate cells of the adult kidney rudiment are organized around a small cavity. Scale bar = 20 μm

c. Cross section of a post-larva at 5 hours after shell loss showing the position of the adult kidney rudiment relative to the intestine. Scale bar = 10 μm

d. Sagittal section of 3 day old juvenile passing through the terminal intestine and the kidney. The internal cavity of the kidney has enlarged. Scale bar = 20 μm

e. Cross section of a 10 day old juvenile passing posterior to the stomach. The kidney has expanded over the entire dorsal surface of the digestive gland. Scale bar = 50 μm

Legend:

ak - adult kidney
an - anus
b - type b larval kidney cell
dg - digestive gland
ey - eyespot
f - foot
i - intestine
mf - mantle fold
n - notum
ol - oral lips
rs - radular sac
s - stomach
sg - salivary gland
vc - velar cells

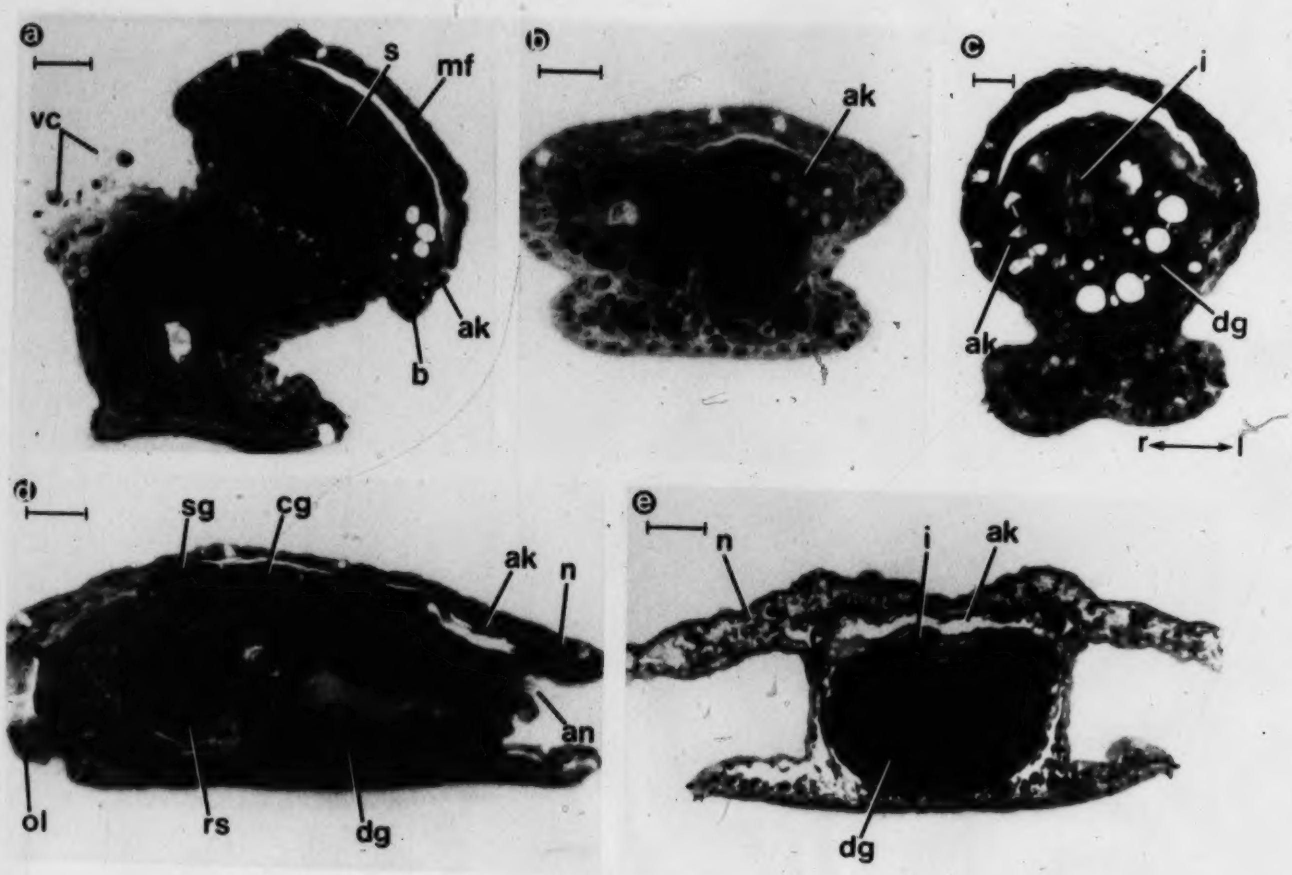

a
vc
s
mf
ak
b
b
ak
c
i
ak
dg
r
l
d
sg
cg
ak
n
an
ol
rs
dg
e
n
i
ak
dg

Figure 34: Development of the gonadal rudiment

a. Sagittal section of a post-larva immediately after shell loss. Note terminal position of the gonadal rudiment. Arrows indicate the larval retractor muscle. Scale bar = 20 µm

b. Sagittal section of a post-larva at 30 minutes after shell loss. The gonadal rudiment is moving under the everted mantle fold. Scale bar = 20 µm

c. Sagittal section of a post-larva at 5 hours after shell loss. The gonadal rudiment is located on the posterio-dorsal surface of the digestive gland, beneath the newly formed notum. Scale bar = 20 µm

d. Sagittal section of a 3 day old juvenile. The gonadal rudiment has moved farther anteriorly. Note the change in the histological characteristics of the germ cells. The cytoplasm is dense and homogeneous and the nucleoli have enlarged. Scale bar = 20 µm

e. Cross section of a 10 day old juvenile showing the proliferation of the cells of the gonadal rudiment. This mass of germ cells is located on the dorsal surface of the digestive gland, posterior to the stomach. Scale bar = 20 µm

Legend:

ak - adult kidney
cg - cerebral ganglion
dg - digestive gland
gr - gonadal rudiment
i - intestine
mf - mantle fold
mpg - metapodial gland
ol - oral lips
ppg - propodial glands
rs - radular sac
sd - dorsal stomach
sv - ventral stomach

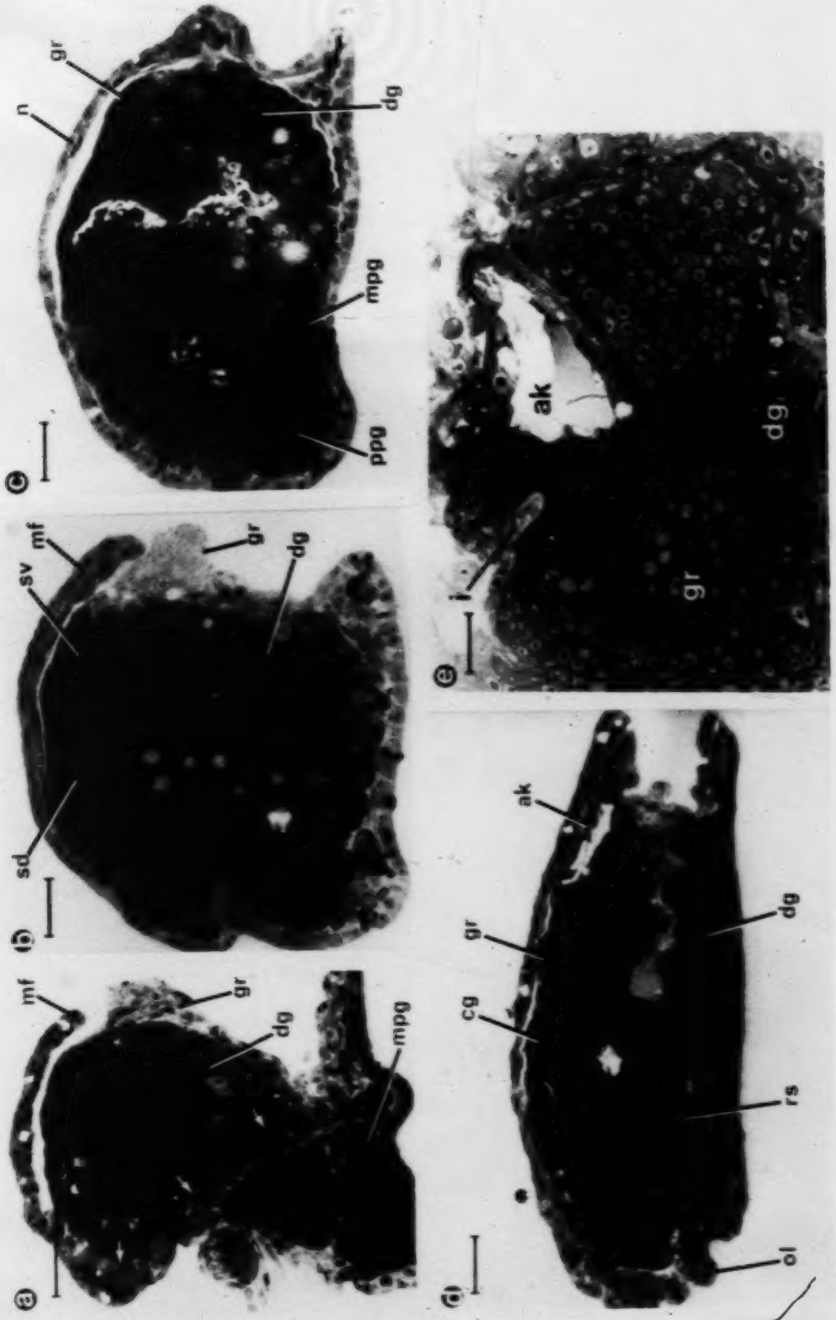

a
mf
gr
dg
mpg
b
sd
sv
mf
gr
dg
c
n
gr
ppg
mpg
dg
d
cg
gr
ak
ol
rs
dg
e
i
ak
gr
dg

Figure 35: Cuticle-secreting cells in the notum of the benthic stage

a. Section through the notum of a 10 day old juvenile showing the cuticle-secreting cell and the newly produced cuticle overlying the epithelium.
Scale bar = 10 µm

b. Same cell type in the notum of an adult.
Scale bar = 10 µm

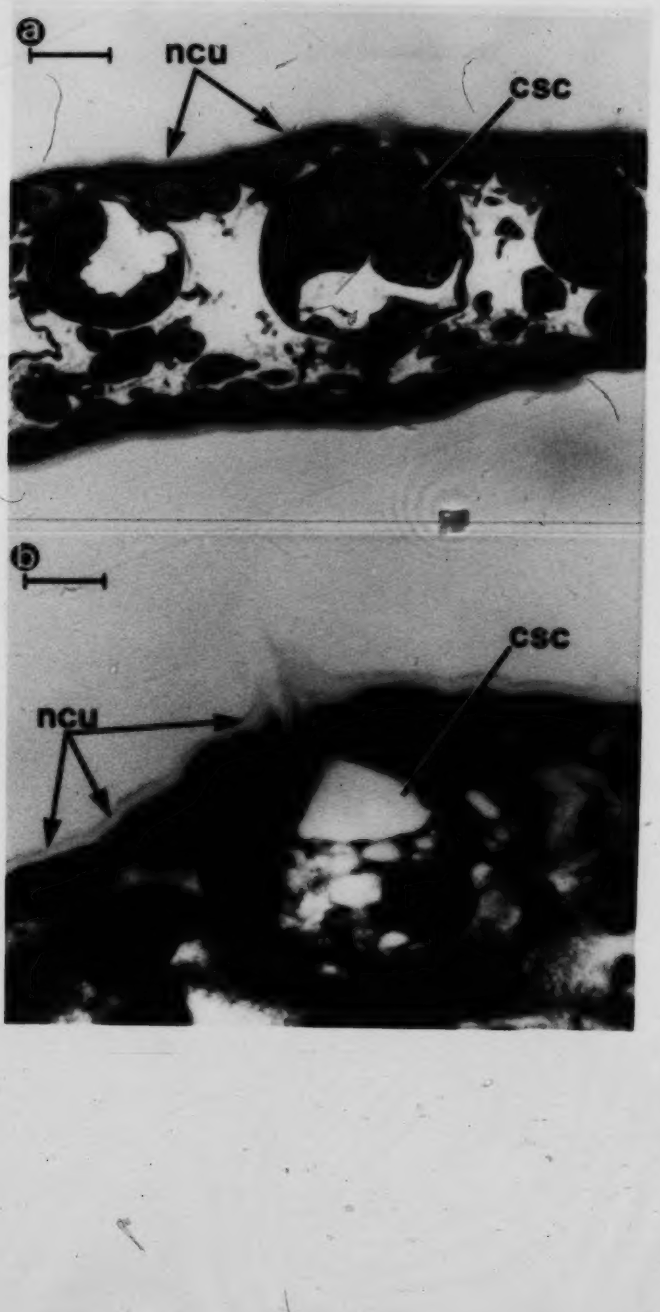

a
ncu
csc
b
csc
ncu

Figure 36: Post-metamorphic growth rate of D. steinbergae. The graph summarizes the data obtained from 8 animals and the bars indicate the range.

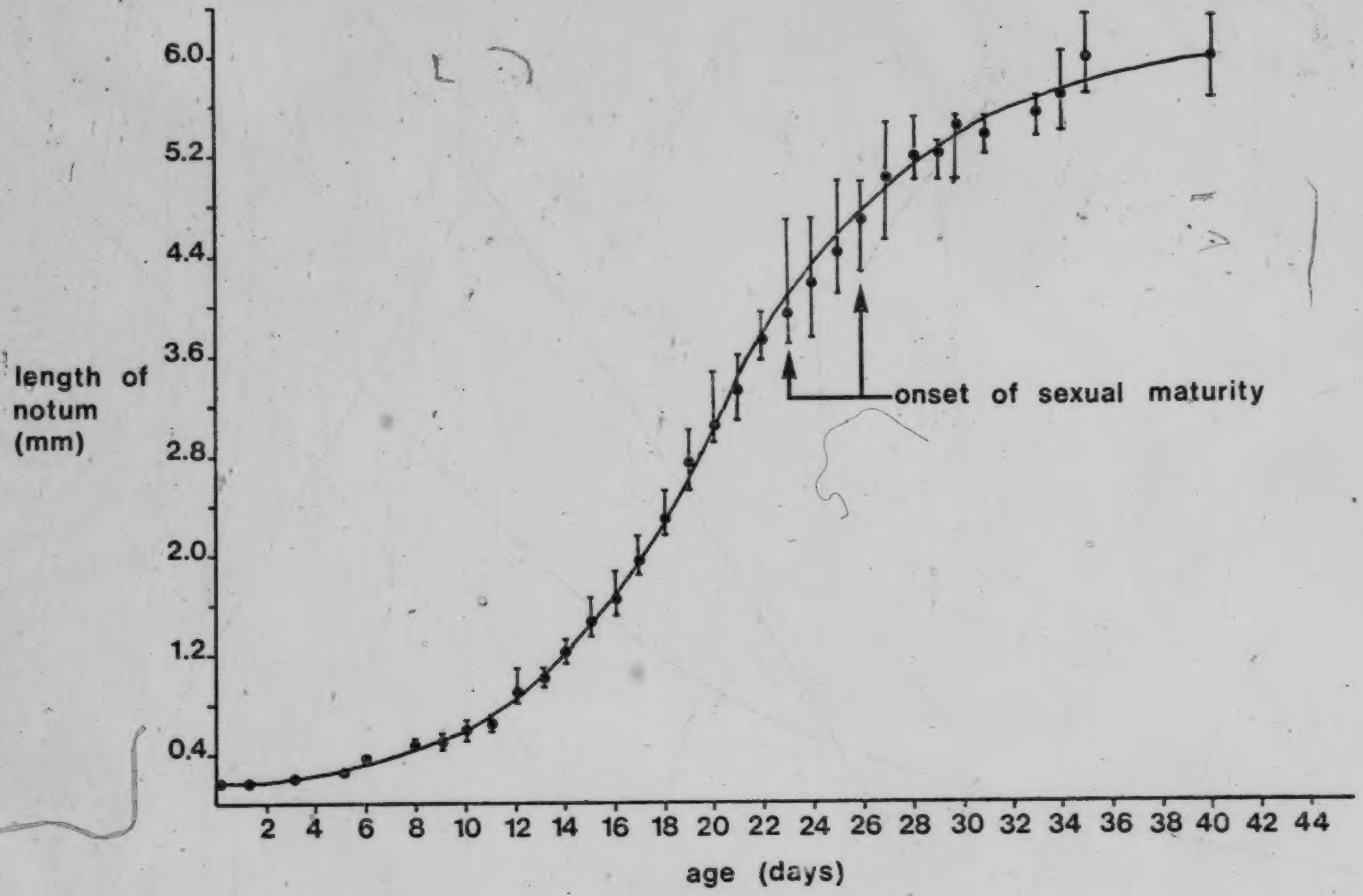

length of notum (mm)
6.0
5.2
4.4
3.6
2.8
2.0
1.2
0.4
onset of sexual maturity
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
age (days)

Figure 37: Successive benthic stages of _D_. _steinbergae_

a. Immediately after shell loss. Scale bar = 0.10 mm

b. 18 hours after shell loss. Scale bar = 0.05 mm

c. 3 day old juvenile. Scale bar = 0.10 mm

d. 8 day old juvenile. Scale bar = 0.10 mm

e. 13 day old juvenile. Note the presence of rhinophores, oral tentacles, and the first pair of branchiae (the latter two structures can be seen through the translucent notum). Pigment begins to appear in the notum at this age. Scale bar = 0.10 mm

f. Two adults on an algal frond encrusted with colonies of _M_. _villosa_. Scale bar = 10 mm

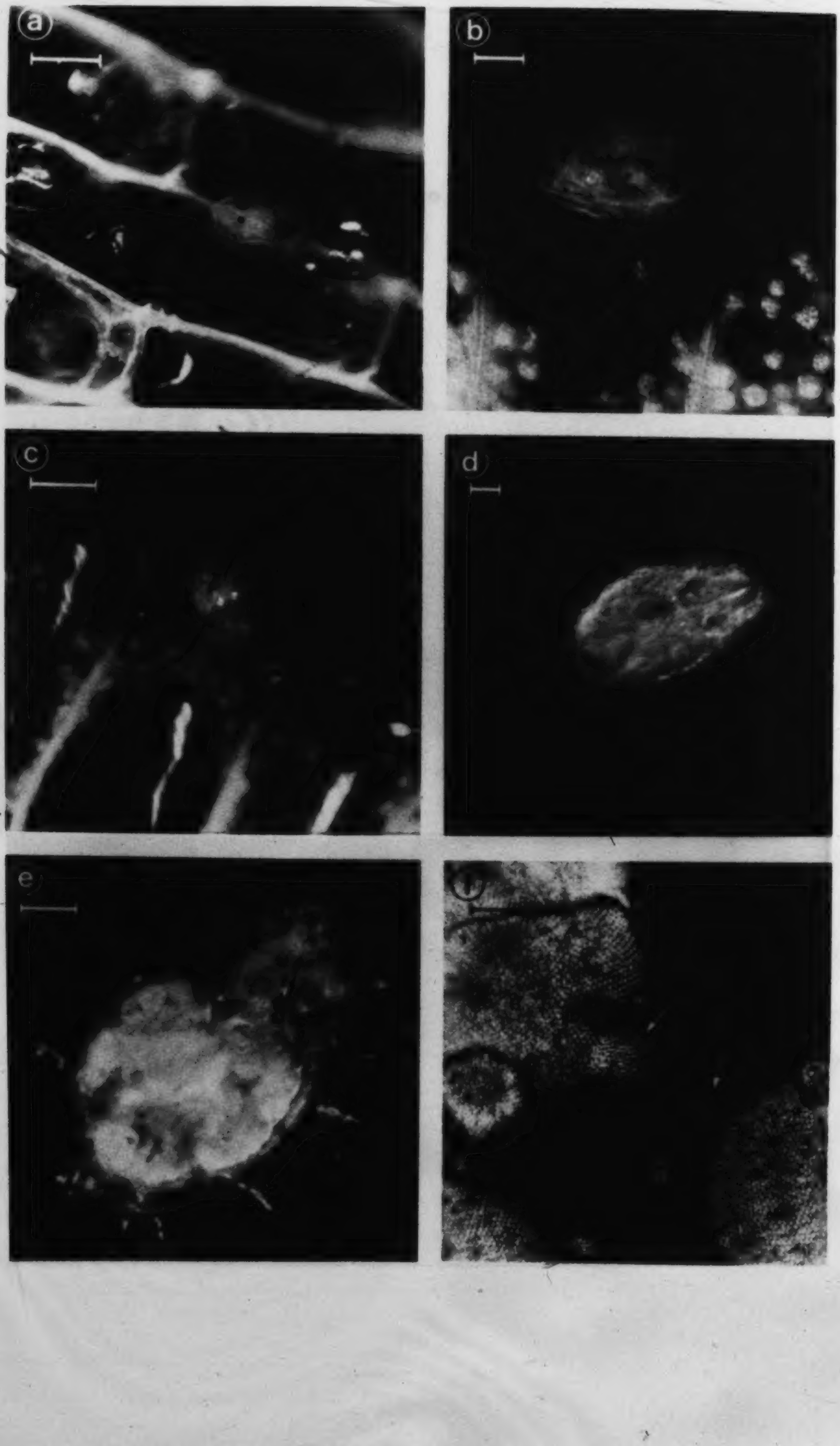

a
b
c
d
e
f

Figure 38: Effect of starvation on juvenile growth rate. Four animals were used in both the control and the experimental group. The bars indicate the range.

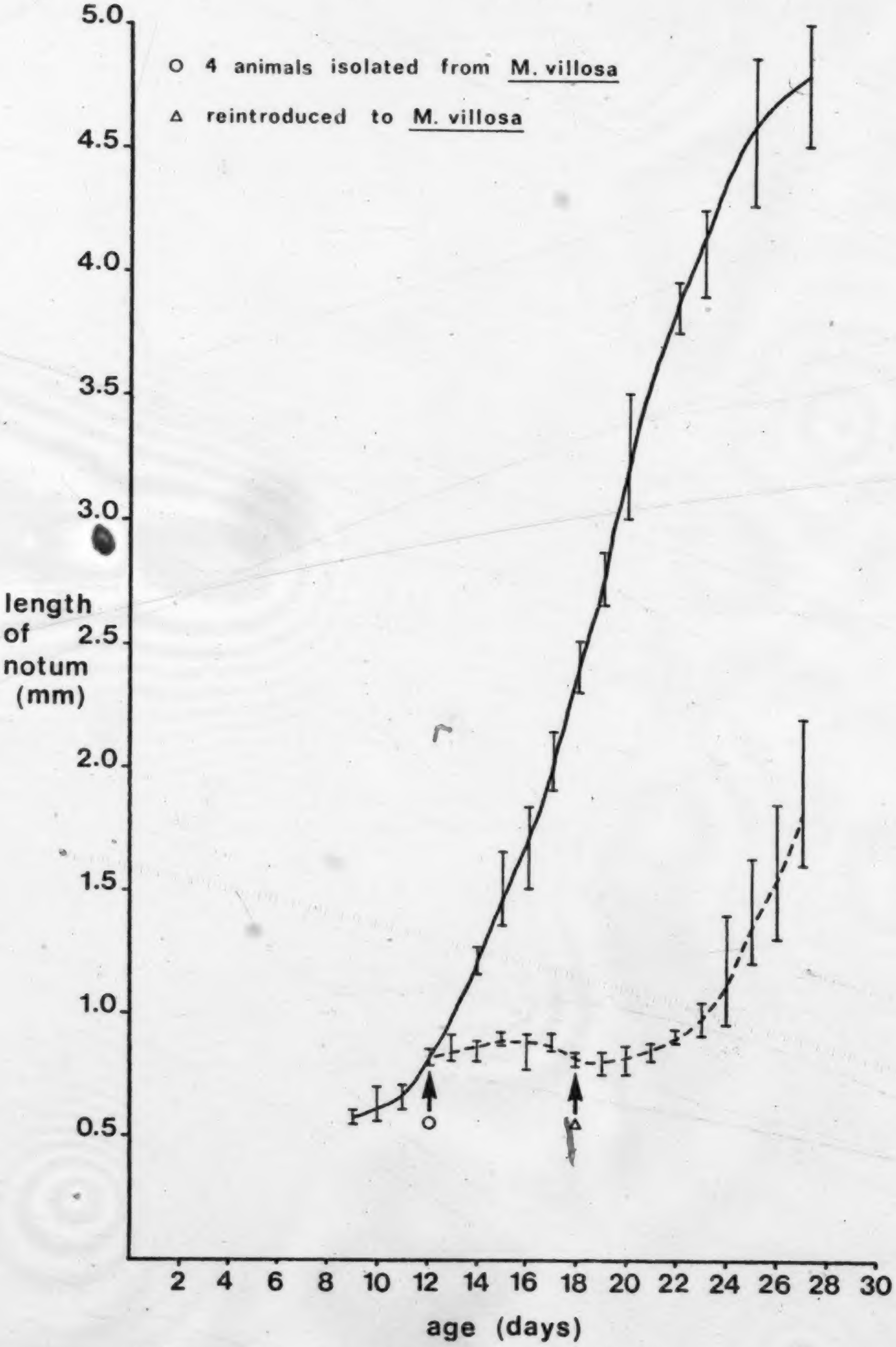

○ 4 animals isolated from M. villosa
△ reintroduced to M. villosa
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
length of notum (mm)
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
age (days)
○
△

Figure 39: Feeding by a young juvenile

a. Photomicrograph of several intact zooids of M. villosa. The bryozoan colony is encrusted on a glass slide. Note that the lophophoral tentacles are clearly distinguishable.

b. Photomicrograph of a 6 day old juvenile feeding on a zooid of M. villosa (the oral end of the juvenile is indicated by the arrow). The structural integrity of the lophophore, in the zooid being attacked, is gone. The dissociated tissue of the zooid is evident throughout the zooecium, although the juvenile remained at the proximal end of the zooid throughout the feeding process. Scale bar = 0.10 mm.

Legend:

a - aperture in the frontal membrane of the zooid
j - juvenile
l - lophophore
zw - zooecium wall
Ez - empty zooecium
Az - zooid being attacked by the juvenile
Iz - zooecium containing an intact zooid

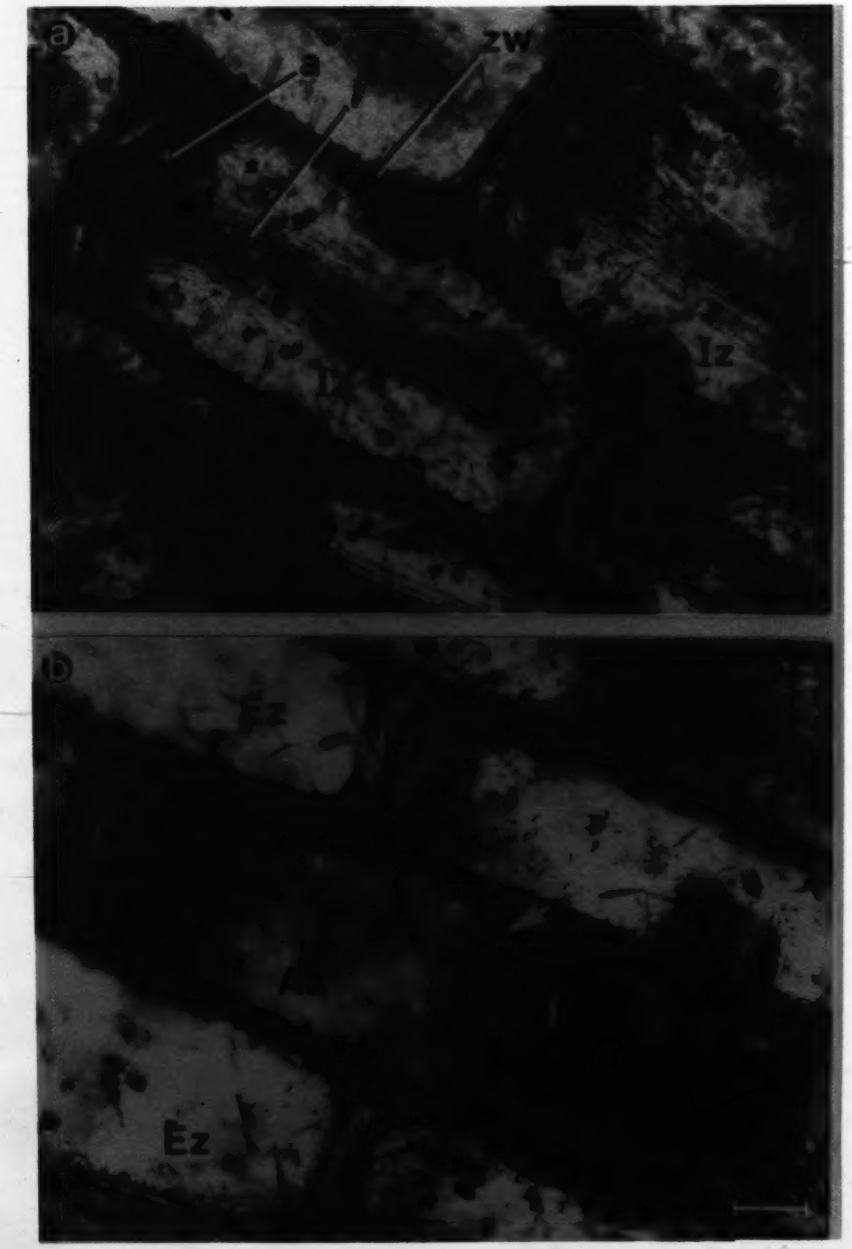
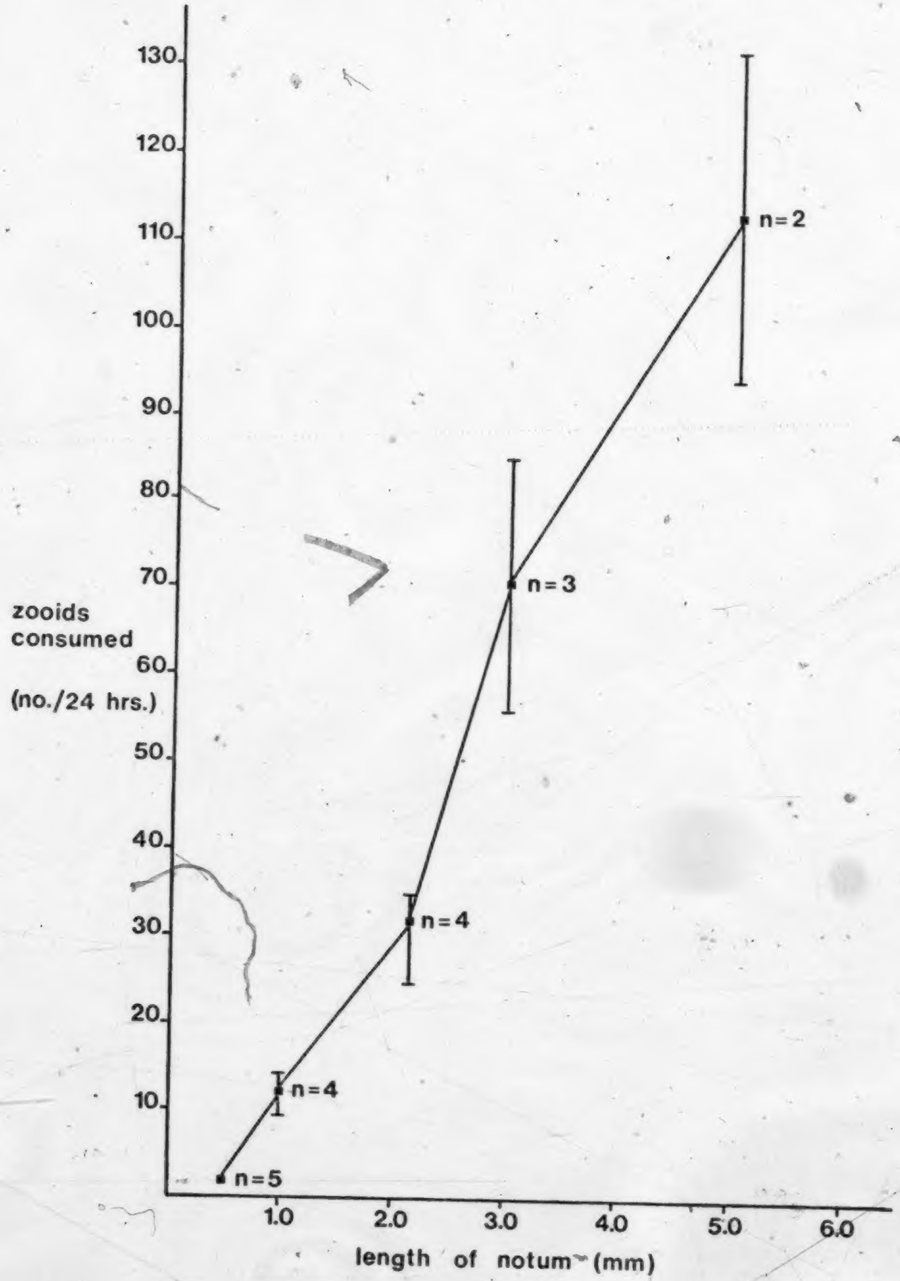

a
a
zw
I
Iz
b
Ez
Ez

Figure 40: Size dependent feeding rate during the benthic phase of D. steinbergae. The number of animals tested in each size class are indicated on the graph (n). The bars indicate the range about the mean.


zooids
consumed
(no./24 hrs.)
130
120
110
100
90
80
70
60
50
40
30
20
10
n=2
n=3
n=4
n=4
n=5
1.0
2.0
3.0
4.0
5.0
6.0
length of notum (mm)

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